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FISH POPULATION ESTIMATES IN SMALL PONDS USING THE MARKING AND RECOVERY TECHNIQUE[1]

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Accurate estimation of the fish population in a body of water is extremely important if fishery resources are to be properly managed and if population dynamics are to be understood. One of the most useful techniques is that of marking a number of fish and estimating the population from the proportion of the marked fish later recovered. This method was first used by C. G. J. Petersen (11) in Denmark but has been widely used in this country in recent years [Underhill, (21); Ricker, (13), (14); Lagler and Ricker, (10); Schumacher and Eschmeyer, (19); Krumholz, (9); Hayes, (7)]. Statistical treatments of the recovery data to determine the best estimate and the standard errors have been developed by Schnabel (18), Schumacher and Eschmeyer (19), and Ricker (16).

In the summer of 1948, the fish populations in ninteen Iowa ponds were estimated by the marking and recovery technique (Table 1). Theoretically the problems of population estimation should be much simpler in small ponds (the largest was three acres) than in large,more complex waters. The condition of the fish population in each pond was first determined by the Swingle method (1). In applying this method a few short hauls were made in the shallow water around the margin of the pond with a common sense seine, 6 to 16 feet long and 4 feet deep. If the fish in the seine consisted of young-of-the-year of both largemouth black bass and bluegills and some two to three-inch yearling bluegills, the pond was classed as being in balance. If no young-of-the-year largemouth bass and very few young-of-the-year bluegills were taken, but the catch was made up almost entirely of the two to three-inch yearling

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[2] Now with the North Carolina Wildlife Resources Commission, Raleigh.

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TABLE 1

DESCRIPTION OF PONDS IN WHICH FISH POPULATION ESTIMATES WERE MADE

Pond No.	Location	Area (Acres)	Secchi Disc (Inches)	General	Record of Stocking		Dates of Estimates	Species not Estimated
					Date	Species		
GROUP 1. Ponds having a balanced bluegill and bass population (On the basis of checks made by the Swingle technique)								
2.......	Lucas Co., Ia., R-20W, T-71N, sec. 4, SW¼	0.6	9	Filamentous green algae and pondweeds	1944	60 bass 1,000 bluegill	6/17-18/48	Green Sunfish, Fathead Minnow
16.......	Marion Co., Ia., R-18W, T-76N, sec. 15, NW¼	0.6	....	Very turbid; Willows and cattails	Not available		7/19-20/48	Carp, Buffalo, Bass, Black Crappie
17.......	Marion Co., Ia., R-21W, T-75N, sec. 11, NW¼	0.4	9	Plankton bloom	1942 1943	bullhead bass; bluegill	7/20-23/48	None
18.......	Marion Co., Ia., R-21W, T-71N, sec. 12, SW ¼	0.2	....	Heavily silted; Cattails and Bulrushes	1943	60 bass 500 bluegill 100 crappie	7/22-23/48	None
33.......	Jefferson Co., Ia., R-8W, T-72N, sec. 1, NW¼	0.6	13	Well fertilized	1945	60 bass 600 bluegill	8/11-12/48	None
36.......	Jefferson Co., Ia., R-8W, T-72N, sec. 13, NW ¼	0.2	6	Heavily silted	Not available		8/9 -10/48	Bullheads, Bass, Channel Catfish, Common Sucker, Bluntnose Minnow

TABLE 1 (*continued*)

DESCRIPTION OF PONDS IN WHICH FISH POPULATION ESTIMATES WERE MADE

Pond No.	Location	Area (Acres)	Secchi Disc (Inches)	General	Record of Stocking		Dates of Estimates	Species not Estimated
					Date	Species		
GROUP 2. Ponds overpopulated with bluegills								
3.......	Wayne Co., Ia., R–20W, T–68N, sec. 31, SE¼	1.5	3	Heavily silted	Not available		6/17–18/48	Bluntnose Minnow Golden Shiner
4.......	Lucas Co., Ia., R–22W, T–71N, sec. 13, SE¼	3.0	4	Shallow water; Heavily silted	1943	300 bass 2,000 bluegill	6/16–18/48	Bass
8.......	Davis Co., Ia., R–12W, T–70N, sec. 4, SW¼	0.1	....	Very turbid; Oil scum on water	1947	100 bass 600 bluegill	6/30/48	Golden Shiner, Channel Catfish, Perch, Carp
9.......	Davis Co., Ia., R–15W, T–68N, sec. 11, NW¼	0.75	2½	Cultivated watershed	1946	75 bass 225 bluegill	6/29–30/48	None
15.......	Marion Co., Ia., R–20W, T–75N, sec. 22, NE¼	0.2	6	Heavily silted	1945	50 bass 750 bluegill	7/20–22/48	None
GROUP 3. Ponds overpopulated with bass.								
34.......	Jefferson Co., Ia., R–8W, T–73N, sec. 31, SW¼	0.3	....	Fertilized Plankton bloom	1945	30 bass 300 bluegill	8/10–11/48	None
35.......	Jefferson Co., Ia., R–10W, T–71N, sec. 5, SW¼	0.2	4	Cattails and Sedge present	1946	20 bass 200 bluegill	8/12–13/48	Crappie, Golden Shiner

TABLE 1 (*continued*)

DESCRIPTION OF PONDS IN WHICH FISH POPULATIONS ESTIMATES WERE MADE

Pond No.	Location	Area (Acres)	Secchi Disc (Inches)	General	Record of Stocking		Dates of Estimates	Species not Estimated
					Date	Species		
GROUP 4. Ponds overpopulated with other species.								
1.......	Lucas Co., Ia., R–21W, T–71N, sec. 12, SE¼	0.5	7	Heavily silted	1937 1943	Misc. fish 50 bass 500 bluegill	6/16–17/48	None
7.......	Davis Co., Ia., R–14W, T–68N, sec. 5, SW¼	0.4	1½	Much shallow water	Not known		7/1 – 2/48	None
19.......	Marion Co., Ia., R–20W, T–76N, sec. 10, NE¼	0.6	....	Turbid	1943	30 bass 25 bullhead 300 bluegill	7/21–22/48	Bass
20.......	Marion Co., Ia., R–18W, T–76N, sec. 25, SW¼	0.3	9	Poisoned after estimates made	1944	bullhead	7/19–20/48	None
31.......	Boone Co., Ia., R–28W, T–83N, sec. 16, SE¼	0.8	....	Heavily silted; Vegetated	1946	80 bass 240 bluegill	8/5 – 6/48	Golden Shiner
32.......	Jefferson Co., Ia., R–8W, T–72N, sec. 30, SW¼	0.4	7	Heavily silted; Very shallow; Poisoned after estimates made	Not available		8/10–11/48	None

bluegills, the pond was classed as being overpopulated with bluegills. When the samples contained no bass young-of-the-year, a few bluegill young-of-the-year, and no two- to three-inch yearling bluegills, an over-population of bass was indicated. If there were no young-of-the-year of either bass or bluegills, but the young or yearlings of other species were present, the pond was classed as being overpopulated with other species.

The common and scientific names of the species of fish found in the ponds are listed below:

Common Name	Scientific Name
German Carp	*Cyprinus carpio* (Lacepede)
Goldfish	*Carassius auratus* (Linnaeus)
Fathead minnow	*Pimephales promelas promelas* (Rafinesque)
Golden shiner	*Notemigonus crysoleucas auratus* (Rafinesque)
Bluntnose minnow	*Hyborhynchus notatus* (Rafinesque)
Common buffalofish	*Megastomatobus cyprinella* (Valenciennes)
Common white sucker	*Catostomus commersonnii commersonnii* (Lacepede)
Channel catfish	*Ictalurus lacustris punctatus* (Rafinesque)
Black bullhead	*Ameiurus melas melas* (Rafinesque)
Tadpole madtom	*Schilbeodes mollis* (Hermann)
Largemouth black bass	*Micropterus salmoides* (Lacepede)
Black crappie	*Pomoxis nigro-maculatus* (Le Sueur)
White crappie	*Pomoxis annularis* (Rafinesque)
Bluegill	*Lepomis macrochirus* (Rafinesque)
Green sunfish	*Lepomis cyanellus* (Rafinesque)
Orangespot sunfish	*Lepomis humilis* (Girard)
Yellow perch	*Perca flavescens* (Mitchill)

All the sampling was done with a 70- by 10-foot, 3/8-inch mesh seine, or with a 60-foot, 3/8-inch mesh seine with a 12-foot bag. Seines were selected for the study since they took larger samples of the fish population in a sohrt time than most other types of gear. Seines also tend to be less selective as to size and species of fish caught. The fish were marked by clipping the left pectoral fin in all species except the bullhead, in which the left pelvic fin was clipped to avoid removal of the pectoral spine. Sampling to obtain fish for marking was usually continued until 20 to 50 per cent of the fish caught were marked. In the smaller ponds, three seine hauls usually sufficed. The measurements and number of each species of fish in each haul were kept separate, because various species differed in vulnerability to sampling.

After the marking operations were completed, no seining was done for at least twelve hours to permit the marked fish to become distributed throughout the entire pond. The same equipment was used to take samples for estimating purposes as was used for marking. Four or five hauls were usually taken for recoveries, varying, of course, with the size of the pond. The measurements and number of marked and unmarked fish of each species were recorded for each haul. From these data the population estimate for each species was calculated.

The Petersen method of population estimation is based on drawing a sample of marked and unmarked individuals from a population containing a known number of marked and an unknown number of un-

marked individuals. The ratio of the number of marked fish recaptured to the total number of fish taken is assumed to be equal to the ratio of the total number of marked fish in the pond to the whole population: that is,

$$\frac{m}{m+u} = \frac{n}{N} \qquad [1]$$

where m is the number of marked fish in the sample, u is the number of unmarked fish, n is the total number of marked fish in the pond, and N is the total population. Since N is the only unknown value in this proportion, it can be determined algebraically. An indication of the accuracy of these estimates can be secured by the application of confidence limits for the binomial distribution to the ratio of marked fish to total number of fish taken in the sample (16). The ratio $m/(m+u)$, is obtained from the sample data, and reference to Clopper and Pearson's (5) chart will give the confidence limits of this ratio. The total population, N, can be estimated as follows:

$$N = \frac{n}{R}, \qquad [2]$$

where n is the known number of marked fish in the pond, and R is the value of the ratio, $m/(m+u)$. By substituting for R the values of its limits, the range of N for 95 per cent or 99 per cent confidence can be estimated. Ricker (13) states that when the ratio of marked to unmarked fish at large is small, less than 0.05, the number of marked fish recovered can be considered a member of a Poisson series. Ricker (12) has tabulated confidence limits for the Poisson distribution. In the present study the ratio of marked fish to unmarked fish was large enough to warrant usage of fiducial limits for the binomial distribution.

A modification of the Petersen method which considers each sample as a separate estimate from which a more accurate estimate is secured by the method of minimizing squares of residuals, has been developed by Schumacher and Eschmeyer (19):

$$N = \frac{S\,[n^2(m+u)]}{S\,(nm)} \qquad [3]$$

where S indicates summation.

Ricker (15) pointed out that the efficiency of [3] is at a maximum when $\frac{n}{N}$ is equal to 0.5. Since, in the population estimates made on the ponds in this study, the proportion of the number of marked fish in the total population was believed to be from 0.2 to 0.5, the Schumacher and Eschmeyer formula was used. Another advantage in

using [3] lies in the fact that Schumacher and Eschmeyer have computed formulae for calculating sampling variance and standard error of the estimate, as follows:

$$s^2 = \frac{1}{k-1}\left[S \left(\frac{m^2}{m+u}\right) - \frac{1\,S\,(nm)}{N} \right] \qquad [4]$$

where s^2 is the sampling variance of the estimated number of fish in the pond and k is the number of samples taken from the pond. It should be pointed out here that a sample was considered to be one drag through the pond with the net used. The standard error of the estimate was calculated by the formula,

$$\sqrt[2]{(N^2)\frac{N\,s^2}{S\,(nm)}} \qquad [5]$$

Certain basic assumptions must hold true before the marking and recovery method can be used to estimate total populations in ponds or other bodies of water (Ricker, 16):

1. The marked fish must become randomly distributed throughout the population.
2. The marked fish are no more or no less vulnerable to the sampling methods than are the unmarked.
3. The marked fish suffer no greater mortality than the unmarked fish.
4. The marked fish do not lose their marks.
5. All marks are recognized and reported on recapture.
6. Recruitment to the population is negligible during the time recoveries are being made.

Since the samples were taken with the seine within one or two days after marking operations, the fish could not have lost their marks in that time. Because the sampling was done shortly after marking, and no pond was connected to another body of water, recruitment to the population being estimated was nil. Finally, since all marks were uniform and each sample was thoroughly checked by one of three persons, it was believed that all marked fish were recognized and reported.

The effect of the marking did not appear to be significant. Only a very few dead marked fish were found, and these consisted principally of white crappies. It is possible that some dead marked fish sank to the bottom, but the fact that so few were recovered leads to the assumption that the fin clipping technique caused little harm to the fish. Ricker (17) demonstrated that fin clipping caused a significant decrease in survival rate in largemouth black bass but not in bluegills. In the present study, the time lapse between marking and recovery was so short as to eliminate errors such as Ricker indicated. Since the ponds were small the seine hauls usually covered most of the pond area, and since the fish were given at least overnight to become redistributed before recapture, it is believed that errors due to nonrandom distribution

of the marked fish were kept at a minimum. Assumption two is the only one on which evidence of validity is not readily available. It will be shown later that in some ponds errors occurred which may have been due to differences in the vulnerability of marked and unmarked fish.

SITUATIONS IN WHICH NO ESTIMATES WERE SECURED

It was not possible to estimate the abundance of certain species either because an insufficient number was marked or no unmarked fish was taken in the recovery samples. For either of these reasons, the following populations could not be estimated in the number of ponds listed: golden shiner, 4; largemouth black bass, 4; bluntnose minnow, 2; carp, 2; channel catfish, 2; fathead minnow, 1; green sunfish, 1; buffalo-fish, 1; white crappie, 1; black crappie, 1; bullhead, 1; common sucker, 1; and yellow perch, 1.

It should be pointed out that it is hardly possible to obtain an accurate estimate of the very small fish of any species. The small fish cannot be handled and marked without a considerable mortality. Furthermore, a large number of the one and two-inch fish can school in a clump of vegetation where sampling operations are hindered; these small fish do not enter the samples, and no reasonable estimate of their numbers is attainable.

In several cases no estimate could be made of the bass population and in other ponds the estimates that were secured had excessively high standard errors. The bass over nine inches in length were often able to avoid capture with the seines and the numbers marked and recovered were too few to make an accurate estimate.

In addition to the nineteen ponds which were studied observations were made on fifteen ponds in which it was not considered feasible to make an estimate. Four ponds had so much growth of aquatic plants and filamentous green algae that it was impossible to sample the populations with the equipment available. The net rolled to such an extent that it was extremely difficult to pull it without damaging the fish. There were trees, posts, and sunken shelters, such as car bodies and brush heaps, in five ponds. No attempt was made to estimate the populations in these ponds, since the danger of entangling the net in these obstructions and tearing or destroying it completely was so great. The fish populations had been recently killed by poison in three ponds, and two other ponds had been stocked only a short time previously; hence there was no purpose in sampling these ponds. The size of one pond made estimating the fish populations unfeasible, because many more samples would have been needed than it was possible to take with the equipment and time available.

POPULATION ESTIMATES AND ERRORS ASSOCIATED WITH ESTIMATES

Table 2 gives the population estimates obtained by the two methods used, the Schumacher and Eschmeyer method of minimizing squares of residuals, and the Petersen ratio estimate. Standard errors of estimate

TABLE 2

Fish Population Estimates

Pond No.	Species	No. of Samples	Schumacher and Eschmeyer Formula					Petersen Ratio Method	
			n	S(m)	S(m + u)	Estimate	Standard Error	Estimate	95% Confidence Limits
GROUP 1. Ponds having a balanced bluegill and bass population.									
2....	Bluegill	5	324	10	121	3,917	1,583	4,050	2,160–8,100
	Bass	5	2	1	2	4	1	4	1–5
16....	Bluegill	3	61	22	88	244	28	244	174–359
	Bullhead	3	40	34	80	94	13	93	75–121
	Green Sunfish	3	8	2	8	32	24	32	23–47
	Orangespot Sunfish	3	7	2	26	91	31	91	80–100
17....	Bass	3	17	11	35	54	6	55	41–77
	Bluegill	3	106	50	151	323	35	321	247–442
18....	Bass	3	5	2	2	5	0	5	....
	Bluegill	3	78	34	71	163	6	163	134–205
33....	Bass	4	8	4	14	29	4	28	21–40
	Bluegill	4	64	8	35	281	69	278	200–427
36....	Bluegill	4	55	50	80	88	5	87	76–104
	Carp	4	3	1	2	7	2	6	5–8
	Green Sunfish	4	41	38	122	126	10	132	100–186

n = number of marked fish in pond.
m = number of marked fish recaptured.
u = number of unmarked fish captured.
S indicates summation.

TABLE 2 (*continued*)

FISH POPULATION ESTIMATES

Pond No.	Species	No. of Samples	Schumacher and Eschmeyer Formula: n	S(m)	S(m + u)	Estimate	Standard Error	Petersen Ratio Method: Estimate	95% Confidence Limits
GROUP 2. Ponds overpopulated with bluegills.									
3....	Bass	3	4	1	2	8	6	8	7–10
	Bluegill	3	78	18	75	325	142	325	236–486
	Bullhead	3	39	4	44	429	178	433	244–975
	Green Sunfish	3	92	24	58	221	51	224	180–297
4....	Bluegill	4	43	9	47	155	19	226	154–358
	Bullhead	4	74	7	49	593	445	529	336–925
	Green Sunfish	4	21	4	24	124	43	124	81–297
8....	Bluegill	2	141	73	174	336	60	336	271–441
	Bullhead	2	517	340	556	845	113	848	728–1014
9....	Bass	5	43	18	41	95	52	95	78–124
	Bluegill	5	133	121	211	219	10	233	199–283
15....	Bass	3	9	7	9	12	1	12	10–13
	Bluegill	3	200	143	251	351	26	351	299–426
	Golden Shiner	3	6	2	9	22	7	27	20–43
GROUP 3. Ponds overpopulated with bass.									
34....	Bass	3	12	8	12	17	3	18	16–21
	Bluegill	3	130	90	142	218	7	206	181–245
35....	Bass	5	3	1	7	21	12	21	14–38
	Bluegill	5	28	18	50	78	21	78	61–104

TABLE 2 (*continued*)
FISH POPULATION ESTIMATES

Pond No.	Species	No. of Samples	Schumacher and Eschmeyer Formula					Petersen Ratio Method	
			n	S(m)	S(m + u)	Estimate	Standard Error	Estimate	95% Confidence Limits
GROUP 4. Ponds overpopulated with other species.									
1....	Bass	3	4	2	11	22	15	22	15–36
	Bluegill	3	140	42	191	637	58	636	451–1,000
	Bullhead	3	30	8	63	236	87	231	142–428
	Goldfish	3	6	1	13	84	80	75	40–150
7....	Bluegill	3	24	10	30	72	10	72	56–100
	Bullhead	3	14	6	18	42	9	42	33–58
	Crappie	3	14	9	11	17	3	17	16–19
	Golden Shiner	3	21	6	8	28	5	28	25–32
19....	Bluegill	3	163	73	159	355	21	354	291–453
	Bullhead	3	29	15	58	114	5	122	81–161
	Crappie	3	102	43	91	216	11	217	179–276
	Orangespot Sunfish	3	5	3	6	10	5	10	8–13
20....	Bullhead	4	216	48	175	769	114	800	584–1,137
31....	Bullhead	4	606	125	257	1,249	170	1,237	1,027–1,554
	Crappie	4	46	21	55	120	35	121	96–164
	Green Sunfish	4	31	8	94	364	360	344	194–775
32....	Bullhead	9	191	82	185	435	30	434	354–562
	Goldfish	9	146	36	140	558	65	562	406–811

and confidence limits are given for the respective methods of population estimation. Other pertinent data concerning the numbers of fish marked, marked and unmarked fish captured, and samples taken are presented. For this study, each pond was numbered in the order in which it was surveyed, and these numbers are used throughout the paper for identifying the ponds. In general, both methods of population estimation gave very similar results. The range of the 95 per cent confidence limits is approximately equal to plus or minus two standard errors. Using the Schumacher and Eschmeyer method of population estimation, one can have only about 67 per cent confidence that the true population lies in the interval of the estimate plus or minus one standard error which in some cases was greater than 75 per cent of the estimate.

The large sampling errors which accompanied many of the population estimates in this study make these estimates insufficiently reliable to serve as a basis for certain management practices. The results of the poisoning operations more forcefully emphasize the fact that the estimates are not as accurate as desired. (Table 3).

The purpose of this study was to investigate the errors which may appear in estimating fish populations. There were two sources of error in this study, one caused by the sampling techniques and the other by characteristics of the fish populations.

In some of the ponds the populations of certain species were too low to obtain estimates. In most of such cases there were probably less than a half dozen fish of each species in a pond. Usually two to three of each species were marked, and no additional unmarked fish were taken in the recovery samples. These low populations were of little concern in regard to how they affected the estimation techniques or the general pond dynamics.

The bass populations, although small in many of the ponds, provided a difficult problem. It was necessary to obtain a reasonably accurate estimate in order to determine what management practices should be applied. In only a few ponds, however, was it possible to secure a reliable estimate. It has been pointed out that bass over nine inches in length were frequently able to swim around the ends or under the net and leap over the float line. Bass were observed to leap over the float line many times, especially while the net was in deep water where the float line could not be held out of the water. As the net was pulled onto shore, the float line was held about two feet above the water, and bass unsuccessfully attempted to leap over the net. There was little that could be done to make netting of bass more efficient with the nets used, because the nets could not be pulled rapidly enough to encircle the bass and prevent them from escaping. The presence of a drainpipe in most of the ponds hindered sampling, and it might help to explain the poor success in capturing bass. Of course the fact that care had to be taken to avoid snagging the net on the drainpipe would make sampling of other species less efficient, too, because the net could not be pulled through the area adjacent to the pipe. Vegeta-

TABLE 3

Comparison of Population Estimates in Two Ponds With the Numbers of Fish Recovered After Poisoning

Pond and Species	Schumacher and Eschmeyer		Petersen Estimate		No. Recovered After Poison	Estimated Population by Poisoning
	Estimate	Standard Error	Estimate	Fiducial Limits		
Pond No. 20						
Bullhead	769	114	800	584–1,137	1,786	1,964
Pond No. 32						
Bullhead	432	30	434	354–562	311	586
Goldfish	558	65	562	406–811	674	906

tion along the shore made landing the net difficult in some ponds, and some fish, including bass, coud have escaped. For any one of the foregoing reasons, or a combination of them, the bass estimates were usually poor.

Low population estimates were obtained for the species of fish in the two ponds poisoned with rotenone (Table 3). The low bullhead estimates can perhaps be best explained by postulating that a group of bullheads remained on the bottom during the time the samples were taken and did not enter the population being sampled. This group could be considered as constituting a separate population inasmuch as none of the group was included in the samples taken for the estimate. For the same reason, if a group of fish was concentrated in a bay where it was not subject to any sampling, the population estimate would not include the fish in the bay and would be an underestimate of the real population. Poisoning would bear this out if recovery were nearly complete.

The bluegill population estimates are believed to be fairly accurate, primarily because the nets used sampled this species more representatively than any other species. Bluegills appeared to be as accessible to sampling one day as the next, and no group of fish of this species was selected more readily than another, as was the case with bullheads and bass.

It is possible that a fish taken in the marking samples is more likely to be taken in the recovery samples than an unmarked fish, because the marked fish is more susceptible to capture than the unmarked fish. Fish able to escape the net once are able to escape it again, whereas, for some reason or another, such as physical condition, other fish are vulnerable to netting time after time. In such a case the effect of marking would probably make the latter even more vulnerable. The estimate obtained would be low, because a proportionately larger number of marked fish than unmarked fish would be taken in the recovery samples. It is difficult to detect, or to correct for, such errors.

A group of fish may be accessible to capture one day and not the next. The fish may stay on the bottom where they are not susceptible to sampling one day, and they may be active in the upper strata of the pond the following day. In larger ponds the fish may be concentrated in one corner of the pond one day and in another part of the pond the next day. In either of these cases the chance for a sizable error of estimate is large when the marking and recovery sampling are done over a period of only one or two days. To correct for the fact that a certain group of fish is vulnerable to sampling one day and not necessarily the next, either because of the habits of the fish or some difference in sampling technique, marking and recovery operations should be carried on over a longer period of time with more samples taken. It appears that each phase of the procedure should be conducted over a period of two or three days in order to obtain more accurate estimates.

Another source of error was the manner in which the nets were

removed from the water. It was not always possible to keep the lead line on the bottom, and fish sometimes escaped by swimming under the net. This was especially true in the case of the goldfish in Pond No. 32 and serves as a possible explanation for the underestimate (Table 3).

A large standard error accompanied many of the estimates primarily because the proportion of marked fish was too small. More marking samples should be taken over a longer period of time to correct for this.

With the net used in this study relatively large numbers of fish could be sampled in a short time, thus making it possible to survey many ponds with a minimum of equipment. However, there are definite limitations as to the effectiveness of the bag net and 70 by 10 feet seine. They are selective in that they sample certain species of fish more efficiently than others. They cannot be used to sample areas around obstructions, such as drainpipes, trees, and sunken scrap. They are difficult to land along a vegetated shoreline. The need for different techniques of capture is readily seen.

POISONING

As a check on the population estimates, all of the fish were eliminated with rotenone from two ponds immediately after the estimates were made. The small ponds afforded an excellent opportunity to make a check, because it was believed that recovery of the poisoned fish was not hindered by depth or expanse. In Pond No. 32, the water was shallow enough to permit wading and picking poisoned fish off the bottom. In Pond No. 20, the net was dragged across the pond to remove poisoned fish near the bottom. Neither pond was connected to any other body of water; the estimates were for an isolated population, and all fish recovered after poisoning represented this same population.

Of 232 bullheads marked in Pond No. 20, 211, or 91 per cent were recovered after poisoning. In Pond No. 32, 90 per cent of the 153 marked goldfish and 86 per cent of the 226 marked bullheads were recovered after poisoning operations. These results indicate higher recovery than has been secured in similar poisoning experiments conducted elsewhere. Ball (2) recovered 59 per cent of the marked bluegills and 45 per cent of the marked trout after poisoning a lake in Ostego County, Michigan, with rotenone. Carlander and Lewis (3) poisoned a small pond in Marion County, Iowa, and recovered the following percentages of marked fish: bluegills, 38 per cent; white crappies, 14 per cent; largemouth black bass, 33 per cent; black bullheads, 80 per cent; and golden shiners, 91 per cent. In recent years, numerous fish populations in ponds and lakes have been poisoned with rotenone, but in only a very few cases were any fish marked before poisoning. It is recommended that a number of fish be marked prior to poisoning in order to obtain information concerning the percentages of dead fish recovered.

Since a high percentage of the marked fish was recovered after poisoning in both Ponds No. 20 and No. 32, it was believed that most

of the unmarked fish were recovered also. The actual number of poisoned fish removed from Pond No. 20 was far greater than the estimated population (Table 3). The same was true for the goldfish and bullhead populations in Pond No. 32; however, the estimates for the latter were not quite as much in error as in Pond No. 20.

In a pond poisoned a year earlier, the bullhead population was similarly underestimated [Carlander and Lewis, (3)]. At that time it was suggested that many of the bullheads were in the bottom mud during the day and not susceptible to capture with the seine. For this reason sampling was done in Pond No. 20 both in the daytime and after dark in the hope that these fish would be more readily caught at night. Forty bullheads were marked in the daytime and 192 at night, and all recovery sampling was done at night. Despite these precautions, the population was underestimated. It would appear that a sizable group of bullheads in these ponds was not vulnerable to capture by seining and regularly eluded capture by burrowing into the mud or hiding in some other manner. Another possible explanation is that once marked the bullheads were more susceptible to capture than the unmarked bullheads.

In seining it was noted that the goldfish reversed directions as the net approached shore and by diving into the loose muck, swam under the net. Most of the goldfish that escaped were apparently unmarked fish, thus giving a high ratio of marked fish in the recoveries and a low estimate of the population.

It is believed that the estimates of the bluegills and other species which are more easily seined are relatively accurate, but further studies on poisoned ponds containing these species are needed. The estimates on bluegills, crappies and golden shiners in the pond reported by Carlander and Lewis (3) compared quite closely with the populations secured by poisoning.

DETERMINATION OF SAMPLE SIZE

The data collected in this study may be used to give some indication of the number of samples needed to estimate a fish population within certain degrees of accuracy.

The total population of a species of fish can be represented T_y. The estimate, $\hat{T}_y$, of the population total can be obtained as follows: [Horvitz, (8)]:

$$\hat{T}_y = \frac{S(Y)}{S(X)} T_x \qquad [6]$$

Where X is the number of the marked fish recaptured in each sample, Y is the total number of fish taken in each recovery sample, and T_x is the number of marked fish in the pond at the time the recovery samples

are taken. It is desirable to know what sample size is necessary for

$$(\hat{T}_y - T_y) \leq d, \qquad [7]$$

with a certain confidence level, such as 95 per cent, where *d* is the difference between the estimated total population and the true total population. The value of *d* is determined by the experimenter, depending upon the degree of accuracy wanted.

$(\hat{T}_y - T_y)$ is assumed to be approximately normally distributed with mean zero and variance, $V(\hat{T}_y)$. Horvitz (3) gives the estimated variance of the ratio estimate as:

$$\hat{V}(\hat{T}_y) = \frac{(\hat{T}_y)^2}{n}\left[\frac{s_X^2}{\bar{x}^2} + \frac{s_Y^2}{\bar{y}^2} - \frac{2\,s_{Y \cdot X}}{\bar{x}\,\bar{y}}\right] \qquad [8]$$

The finite population correction term $(N-n)/N$, is ignored, because *n*, the sample size, is small relative to *N*, the number of sampling units; that is, $(N-n)$ is approximately equal to *N*. The number of sampling units, *N*, approaches infinity when sampling with replacement, as was the case in nearly all of the ponds in this study. In [9] s_x^2 and s_y^2 are the sample variances, and Sy.x is the sample covariance. All of these values can be determined from pre-sample data.

Squaring both sides and dividing by $\hat{V}(\hat{T}_y)$, formula [7] becomes

$$\frac{(\hat{T}_y - T_y)^2}{\hat{V}(\hat{T}_y)} \leq \frac{d^2}{\hat{V}(\hat{T}_y)} \qquad [9]$$

Assuming that $(\hat{T}_y - T_y) / \sqrt{\hat{V}(\hat{T}_y)}$ is approximately distributed as "Student's" "t" formula ["Student," (20)], [9] can be written as follows:

$$t^2 \leq \frac{d^2}{\hat{V}(\hat{T}_y)} \qquad [10]$$

Substituting the expression for $\hat{V}(\hat{T}_y)$ in [10], it can be seen that

$$\frac{(\hat{T}_y)^2}{n}\left[\frac{s_X^2}{\bar{x}^2} + \frac{s_Y^2}{\bar{y}^2} - \frac{2\,s_{Y \cdot X}}{\bar{x}\,\bar{y}}\right] \leq \frac{d^2}{t^2} \qquad [11]$$

$$\text{and } n \geq (\hat{T}_y)^2 \left[\frac{s_X^2}{\bar{x}^2} + \frac{s_Y^2}{\bar{y}^2} - \frac{2\,s_{Y \cdot X}}{\bar{x}\,\bar{y}}\right] \frac{t^2}{d^2} \qquad [12]$$

The value for *t* is based on the number of degrees of freedom in the pre-sample at the desired confidence level and can be obtained from

a table presented by Fisher (6). The sample size, n, is the number of samples required to obtain an estimate of the total population within the limits of d fish with a confidence coefficient equal to the confidence level of t.

As an example, the calculations necessary to determine the number of samples to estimate the goldfish population within 100 fish with 95 per cent confidence are given below:

	Sample Number									Total	Mean
	1	2	3	4	5	6	7	8	9		
Marked Fish Recaptured, X....	2	0	2	1	2	1	3	11	14	36	4
Total Fish in Sample, Y.......	8	2	11	5	14	6	14	37	43	140	15.55

Number of marked goldfish, $T_x = 146$

$$s_x^2 = S(x^2)/(n-1) = 196/8 = 24.5$$
$$s_y^2 = S(y^2)/(n-1) = 1682.2225/8 = 210.28$$
$$s_{Y \cdot X} = S(xy)/(n-1) = 568/8 = 71$$

$$\hat{T}_y = \frac{S(Y)}{S(X)} T_x = \frac{140}{36} (146) = 562$$

$t_{.95}$, 8 degrees of freedom $= 2.306$

$$n \geq (562)^2 \left[\frac{24.5}{(4)^2} + \frac{210.28}{(15.55)^2} - \frac{2\ (71)}{(4)\ (15.55)} \right] \frac{(2.306)^2}{(100)^2} \geq 20$$

These results show that less than one-half the necessary number of samples was taken. This method of determining sample size gives reasonable assurance that the population will fall in the designated limits.

The number of samples needed varies greatly in the various ponds and with the different species of fish (Table 4). Part of this variability may be due to not taking sufficient samples to estimate the variance accurately enough for the determination of the number of samples needed. The variance of the ratio estimate is only an approximation, and its degree of accuracy depends greatly upon the number of samples taken. The variance of the ratio estimate is generally an overestimate when too few samples are taken.

It is obviously impractical to try to secure an estimate within 10 per cent of the true population in many of the ponds using the methods of this study. As the degree of accuracy required is decreased, however, the number of samples needed is decreased in geometric proportion (Table 5).

When any other method of population estimation, such as that

TABLE 4

Number of Samples Required To Estimate Fish Populations With Less Than 10 Per Cent Error With 95 Per Cent Confidence in Ponds Included in This Study

Pond Number	Species				
	Bass	Bluegill	Bullhead	Crappie	Green Sunfish
1	2,356	50	78	....	....
2	....	408	....	....	....
3	2,676	393	818	....	260
4	....	102	408	....	124
7	....	134	170	137	....
8	....	989	523	....	....
9	....	20	257	....	....
15	....	132	24	....	....
16	....	76	82	....	....
17	70	41	....	....	....
18	....	7	....	....	....
19	....	13	5	11	....
20	....	....	93	....	....
31	....	....	88	51	2,196
32	....	....	13	....	....
33	228	168	....	....	....
34	164	49	....	....	....
35	2,601	164	....	....	....
36	....	38	....	....	74
Median	1,292	89	90	51	192

developed by Schumacher and Eschmeyer, is used to evaluate the number of fish in a body of water, the variance of the estimate can be used to determine sample size necessary to obtain an accurate estimate. For example, Schumacher and Eschmeyer's standard error can be used to determine sample size k, where $\hat{N}$ is the estimate of the total population N. It is desirable to know how many samples are needed so that

$$(\hat{N} - N) \leq d \qquad [13]$$

where d is a chosen interval and $(\hat{N} - N)$ is assumed to be normally

TABLE 5

Number of Samples Required To Estimate Fish Population Included in This Study With 95 Per Cent Confidence

Percentage Error Permitted	Species				
	Bass	Bluegill	Bullhead	Crappie	Green Sunfish
10	1,292	89	90	51	192
20	323	22	22	13	48
30	145	10	10	6	21
40	81	6	6	3	12

distributed with mean zero and variance $V(\hat{N})$. The estimated variance is as follows,

$$\hat{V}(\hat{N}) = \sqrt{\hat{N}^2 \quad \frac{\hat{N}_s{}^2}{S(nm)}} \qquad [14]$$

where s^2 is as given in [4].

After squaring both sides of [13] and dividing through by $\hat{V}(\hat{N})$, and assuming that $(\hat{N} - N)/\sqrt{\hat{V}(\hat{N})}$ is approximately distributed as "Student's" "t," [13] can be written as follows:

$$t^2 \leq \frac{d^2}{V(N)} \qquad [15]$$

Substituting the expression $\hat{V}(\hat{N})$ in [15], it is seen that

$$\hat{N}\sqrt{\frac{\hat{N}\left(\frac{1}{k-1}\right)\left[S\left(\frac{m^2}{m+u}\right) - \frac{S(nm)}{N}\right]}{S(nm)}} \leq \frac{d^2}{t^2} \qquad [16]$$

Clearing the radical in [16] and transposing, the sample size, k, is shown to be

$$k \geq \frac{\hat{N}^3\left[S\left(\frac{m^2}{m+u}\right) - \frac{S(nm)}{\hat{N}}\right]}{\left(\frac{d^2}{t^2}\right)^2 \quad S(nm)} + 1 \qquad [17]$$

Sample sizes using formula [17] have not been worked out for all of the ponds included in this study, but for the one or two cases for which *k* has been determined, the number of samples required for a population estimate with 10 per cent error and 95 per cent confidence were less than that already taken. It should be pointed out that whenever the estimated variance of any population estimate is inaccurate or is biased, the resulting value arrived at for the necessary sample size will be misleading.

Of course, all of these calculations are based on the assumption that the fish are being randomly sampled. If the sample is biased (e.g., if it is selecting from one group of fish, with another group of fish avoiding capture), increasing the number of samples may not result in a more accurate estimate of the population.

SUGGESTIONS FOR ESTIMATING FISH POPULATIONS IN SMALL POND

The most serious problems encountered in the estimation of the fish populations in these ponds was the difficulty of getting random

samples of the fish. In several cases there were indications that some of the fish were more susceptible to capture than others and since these were the fish most apt to be marked and recovered, the population estimate tended to be low. It is recommended that several methods be used to capture the fish (e.g., seines, hoopnets, gillnets, and angling) so that the effect of the selectivity of each type of gear be minimized. A measure of the relative efficiency of each type of sampling gear can be obtained at the same time. It is also suggested that the marking and recovery operations be extended over several days, probably up to about two weeks, so that if certain groups of fish remain in limited areas for a period of time there will be more opportunity for random distribution of the marked fish. As the period of operations is extended, however, errors due to differential mortality of marked fish and to recruitment increase.

After several samples have been taken from a pond, the data should be analyzed after the fashion previously described to determine the number of samples needed to secure the required accuracy.

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BOB-WHITE QUAIL NESTING AND PRODUCTION IN SOUTHEASTERN IOWA [1]

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From January, 1946, to April, 1949, an intensive study was conducted on the Interior Bob-White Quail, *Colinus virginianus mexicanus* Linnaeus, on the Eldon Research Area, located in Salt Creek Township, Davis County, Iowa. The Research Area, a 1,600-acre tract, was composed of 620 acres of state-owned land and 980 acres of privately-owned land, which bordered the state area on all sides. The entire area was open to hunting in season. The purpose of the study was to obtain data on the response of the bob-white to land management practices and to install a planned management program. As a part of these studies, three years of nesting and production data were gathered.

In topography the Research Area was largely upland, with about one-fourth lowland bordering Soap Creek along the northern portion. Two-fifths of the upland supported dense stands of oak-hickory, *Quercus-Carya*, timber. The remainder was largely in permanent pastures and small, irregular, cultivated and idle fields. Lowland areas were intensively cultivated with the fields small, irregular, and bordered by mature stands of trees and shrubs.

The initial survey (1946) indicated that the state-owned portion was extremely unproductive from an agricultural standpoint, largely due to improper land management practices. There was little or no evidence of crop rotation, controlled grazing, or soil conservation; whereas on the bordering lands such practices were conspicuous by contrast. As a result the better bob-white habitats were found on the border strip. In 1947 and 1948 a planned farm program was initiated on the tillable state land and, except for twenty acres in 1947, no livestock were on these lands. Dense stands of cover (grasses and weeds) developed in the ungrazed pasture areas by the fall of 1948. Observations indicated that this cover development in 1947 and 1948

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[1] The research was under Dr. T. G. Scott, former leader of the Iowa Cooperative Wildlife Research Unit and his successor, Dr. E. L. Kozicky, and Dr. G. O. Hendrickson, Department of Zoology and Entomology, Iowa State College, Ames, Iowa. Appreciation is acknowledged for the helpful guidance and sympathetic interest of each.

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was less suitable to nesting birds than the overgrazing in 1946 but was attractive to winter coveys (5). Along with the farm program various developmental and management practices were initiated which likewise influenced quail habitats on the state area.

The weather conditions during the investigation were variable and somewhat abnormal but less so in 1949 than during the two previous years. Rainfall was record-breaking during June of 1946 and 1947 and resulted in floods which covered much of the lowland on the area on two occasions each year. Drouth followed these heavy rains and was particularly severe in 1947 when no rainfall was recorded on the area for July, August, and the first two weeks in September. The nesting data for 1946 and 1947 appeared to reflect these climatic adversities.

TECHNIQUES OF INVESTIGATION

Continuous observations were made in residence from March 26 to December 15 in 1946 and from March 26 to September 4 in 1947 and 1948. As time permitted, trips were made to the area when not in residence.

Censuses were made three times each year to determine the quail populations and rates of production. The first was made prior to the spring dispersal (March 28–April 1) to establish the potential seed stock and wintering loss; the second, to determine the per cent of summer gain, was made in mid-November just before the hunting season (November 13–15); and the third, to establish posthunting population, was made shortly after the hunting season closed (December 26–29). In censusing the direct enumeration technique, used by Errington and Hamerstrom (3), was employed.

After the spring dispersal began, data were obtained on the number of pairs and the tentative ranges. Following the localization of pairs a deliberate effort was made to locate their nests by the strip and quadrat methods. With the former, the prospective nesting areas were traversed in parallel six- to seven-foot adjoining strips with the aid of a walking stick to open up the vegetation. Ninety-one per cent of all known nests were found in this manner. In using the latter method, quadrats of 1,089 square feet each, were established in a linear fashion and each was thoroughly searched. Nine per cent of the nests found were located as a result of this technique. The quadrat procedure was of the greatest value when working the heavier cover, such as occurred along field edges, creek banks, roadsides, or fence rows, whereas the strip method was found best for examining field interiors. An attempt in midsummer, 1947, to find nests with the aid of a bird dog proved unsatisfactory.

The range of each pair was visited repeatedly until a nest was found or until it became evident by the activities of the pair that no nest existed. Every precaution was taken to avoid disturbance of the pair when a nest was located. Dates of nest commencement were established on the basis of number of eggs, stage of nesting, and age

of remaining "sign" if the nest had hatched, been destroyed, or abandoned. Determination of causes of nest destruction or abandonment depended on the accuracy of "sign" interpretation. Unless the causes of nest losses were definitely indicated by "sign," they were classed as undetermined. Nests were visited every third day, unless hatching was near, and then more frequent observations were made.

RESULTS

NESTING

As a result of prenesting censuses, the following breeding populations were estimated: 1946, seventy-two birds; 1947, forty-eight birds; 1948, sixteen birds; and 1949, fifty-seven birds. The total number of nests found during the three years was forty-six—sixteen in 1946, eighteen in 1947, and twelve in 1948. Nesting data were not gathered in 1949.

NESTING SEASON

The earliest established nest found in 1946 was discovered on April 20, in 1947 on May 1, and in 1948 on April 24. The latest nest known in 1946 was found on July 15, in 1947 on August 1, and in 1948 on August 18. Paired birds were still in evidence following the termination of the nesting studies (August 20) all three seasons. This was particularly true in 1947 when seven pairs were under observation, five of which continued to behave as nesting birds on September 1. A brood of fifteen was estimated to be a week old when seen on September 24, 1946. The nest from which these young came was probably established during the first half of August. Four of the ten coveys found while making the prehunting census during November 13 to 15, 1947, consisted primarily of birds not more than eight weeks old; and in one of them the birds were not more than five or six weeks old. During the same period in 1948, a covey of six was estimated to be less than six weeks in age. Such young birds at this time indicate that nests were started during the latter part of August and early September. The nesting season, therefore, was spread over approximately 115 days in 1946, 135 days in 1947, and 141 days in 1948.

A major and minor peak in nest commencement was evident for the three seasons (Fig. 1). The major peak in 1946 occurred during May 1 to 15 and the minor peak during June 16 to 30; in 1947 they were May 16 to 31, and June 16 to 30, respectively; and in 1948, May 1 to 15 and June 16 to 30, respectively. Because the initial nesting began in May, the first peak was probably normal. The peak occurring in June was believed indicative of the heavy nest loss in late May and early June (Table 1).

As shown in Figure 1, the first nesting peak occurred two weeks earlier in 1946 and 1948 than in 1947. Considering the time of the commencement of the spring break-up for the three years (1946, March

2; 1947, April 16; and 1948, April 3), the earliest nests and nesting peak could be expected in the 1946 and 1948 seasons. The 1948 nesting was more evenly distributed throughout the season than in 1946 and 1947 (5).

COVER UTILIZATION

Thirty-six (78.3 per cent) of the 46 nests found were located on nonproductive lands (lands showing no potential economical returns such as roadsides, fence rows, field edges, and idle land). Only 25.0 per cent were successful. These data concur with the findings of Moorman (7).

Twelve of the thirty-six nests located on nonproductive lands were in the lowlands where intertilled crops were predominant. Of the remaining twenty-four found in the uplands, seventeen were closely associated with cropped areas. In other words, the location of twenty-

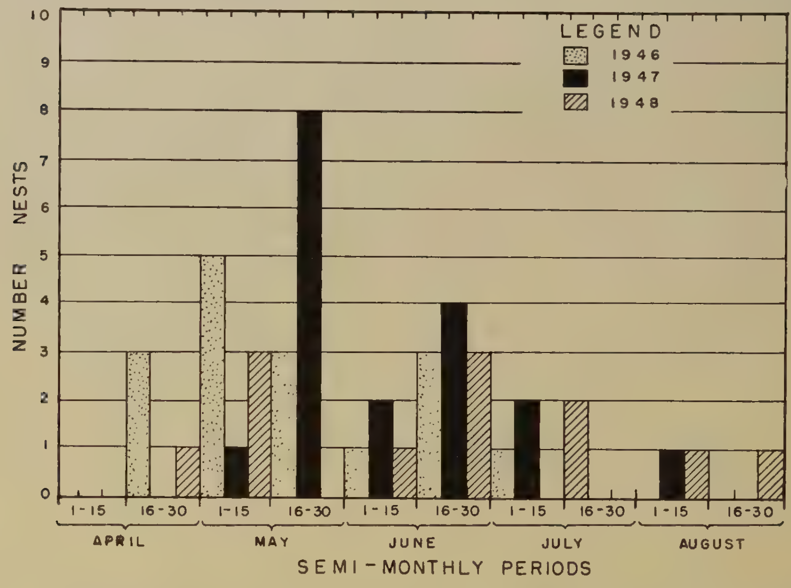

FIG. 1.—Commencement of bob-white nests on the Eldon Research Area, 1946, 1947, and 1948.

nine of the thirty-six nests on nonproductive lands was probably directly influenced by association with crop lands. Inasmuch as the heaviest winter populations were in and around tilled lands and initial nests were in or near these winter ranges, it could be assumed that such an association was to be expected. However, the attractiveness of these sites was evident in the continued nesting as the season advanced, even when other areas appeared more suitable. Indications were that the

distribution and density of cover may have been the important factors. The seven nests not associated with farmed areas were all started before May 5, at a time when current plant growths were at a minimum and ecotones not so evident. Further, areas of dense plant growth, such as idle fields and grassed waterways, were never found to be used for nesting or rearing the young except at the outer edges. This seems to indicate that the "edge effect" provided by numerous farmed areas was attractive to nesting pairs.

Roadsides had the highest nest density (one to 1.7 acres) but

TABLE 1
Semimonthly Nest Losses, 1946–1948

Year	May 1–15	May 16–31	June 1–15	June 16–30	July 1–15	July 16–31	Aug. 1–15
1946	2	1	4	1	1	1	1
1947	0	2	7	1	1	1	2
1948	0	1	4	1	......	1	1
Total	2	4	15	3	2	3	4

the lowest percentage (16.7) of success. In contrast, idle land had the lowest density (one to ten acres) but the highest percentage (35.7) of success in the nonproductive lands. Fence rows and field edges were intermediate in these respects, even though the larger number of nests was found there.

The productive lands (pasture, farm lot, crop land, and timber) supported ten of the forty-six nests (21.7 per cent), and nests on them were 40.0 per cent successful. Nest densities were low, but of the 1,360 acres in productive lands, less than one-half could be considered potentially suited for nesting. Pastures contained all ten nests found in productive lands; the open type yielded eight and the woodland type two. Farm lots and fields utilized for hay or grain were devoid of nests. Other nesting studies—Stoddard (8), Errington (1), Moorman (7)—have shown some utilization of these lands, particularly hay fields. No nests were found in timbered areas.

It was difficult to determine what factors in the environment were the most influential in choice of nesting sites. There was possibly some association between presence of nests and such factors as drainage, sources of water, available escape and nesting cover, and openings resulting from roads, gullies, and clearings. Each is discussed in an attempt to evaluate its significance with respect to the distribution of nests.

Of all nesting sites, forty (87 per cent) were adjudged as well drained, five (11 per cent) as fair, and one (2 per cent) as poor. Ratings were made on the basis of the subjection of nests to overflow as the result of normal rainfall.

As the nesting season advanced, nests were found progressively farther from sources of water (Fig. 2). The distance of the nests from

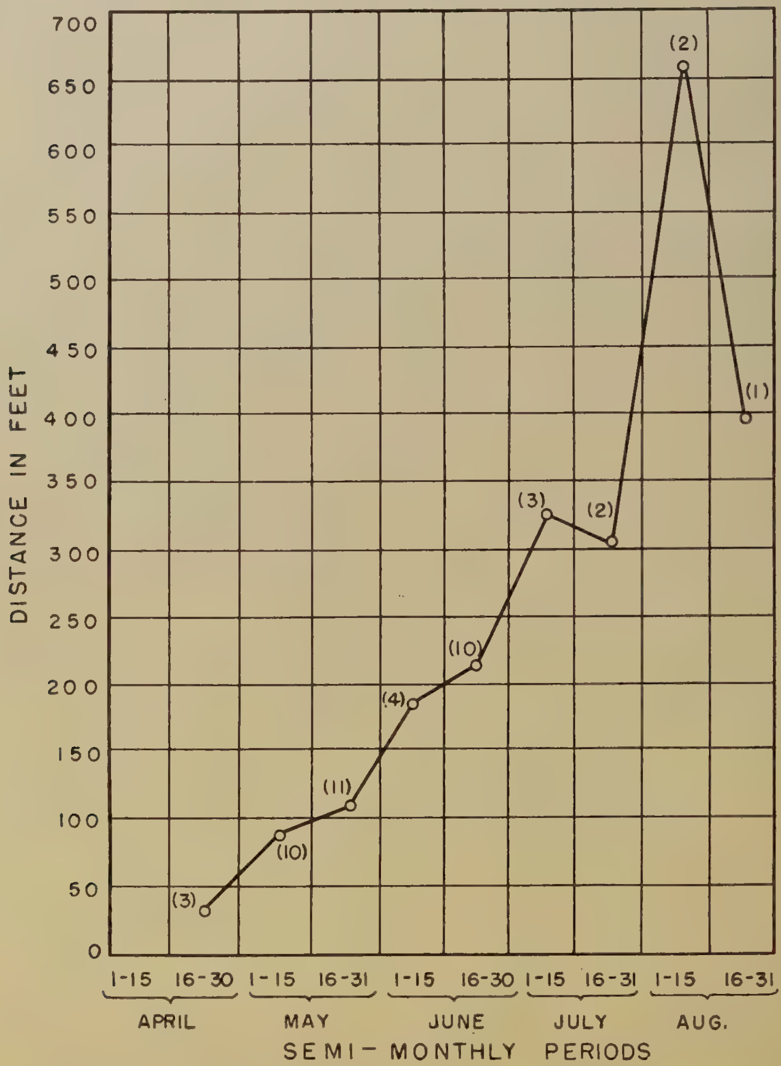

FIG. 2.—Relationship of the distance of bob-white quail nests from a permanent source of water and the time of the year.

water varied from 3 to 963 feet, the average distance for the forty-six being 172.4 feet.

Twenty-eight (61 per cent) of all nests were either in or at the edge of woody cover sufficient to furnish good protection. Mean distance of the remaining eighteen (39 per cent) from this type of cover was 139.5 feet with a range of 21 to 315 feet.

Forty-four (95 per cent) of the forty-six nests were constructed of and situated in grasses. Only plant growths of previous years were used in the nest structure. Current growths were believed employed primarily for the protective cover that they afforded the site. Twenty-three (50 per cent) nests were located in pure stands of grass. The others were in mixed plant growths which consisted largely of grasses. Bluegrass, *Poa pratensis,* red top, *Agrostis alba,* timothy, *Phleum pratense,* and poverty grass, *Aristida hiemalis,* were the principal grasses used. Of these, bluegrass was used in thirty-six nests. Seventeen (38 per cent) of the nests were situated at the base of other plants ranging in height from two to twelve feet. These plants were American elm, *Ulmus americana;* coral berry, *Symphoricarpus orbiculatus;* white ash, *Fraxinus americana;* honey locust, *Gleditsia triacanthos;* mullein, *Verbascum Thapsus;* wild lettuce, *Lactuca canadensis;* and multiflora rose, *Rosa multiflora.*

Nests appeared to be closely associated with breaks in the cover pattern afforded by field edges, roadways, clearings, plots of idle land, and gullies. The mean distance of forty-five nests from a cover edge was eleven feet with a range of zero to sixty-two feet. Of these, twenty-two nests were at a distance of four feet or less. One nest failed to show any association with cover edge. Field data indicate a steady decrease in distance from cover edges from April to early June; after this time mean distances were relatively the same.

CLUTCHES

The number of eggs in twenty-four completed clutches varied from ten to twenty-two with a mean number of 15.04 per nest. Indications were that the larger clutches occurred among the early nests (April and May). But, a marked decline as the season progressed was not so pronounced as that reported by Lehmann (6), Moorman (7), and Errington (1).

During the three seasons, 169 young were produced from 184 eggs in thirteen successful nests. In 1946, fifty-nine of sixty-two eggs hatched (five nests), a 95.6 per cent hatching success; in 1947, fifty-one of sixty-two eggs hatched (four nests), an 82.3 per cent hatching success; and in 1948, fifty-nine of sixty eggs hatched (four nests), a 98.03 per cent hatching success. The 1947 results were probably below the average because of the adverse climatic conditions during the entire nesting season. Rate of success for the three years combined was 91.8 per cent which is higher than reported by Stoddard (8) and Lehmann (6).

NEST LOSSES

In this study 28.3 per cent of all nests were successful which was

comparable with the findings of Moorman (7) in south central Iowa. Table 2 presents the causes of nest losses occurring during this study. Slightly more than 30 per cent of the unsuccessful nests were lost because of abandonment as a result of disturbance to the birds or their nests. Climatic factors, which accounted for 21.3 per cent of this type of desertion, manifested their effects only in 1946 and 1947 when 10.63 and 12.09 inches of rain fell, respectively, in June. In 1948, when the rainfall was near normal, no nests were believed deserted because of climatic factors. Predators accounted for 57.6 per cent of the nest losses during the three years. Snakes were abundant and accounted for 12.2 per cent of the losses. Next in importance were feral cats and red foxes. Fifteen per cent were lost to undetermined causes of predation.

BROODS

Data were obtained on eighteen broods and of these thirteen were

TABLE 2

Causes of Bob-White Nest Losses on the Eldon Research Area, 1946, 1947, and 1948

Cause of Loss	1946	1947	1948	Totals	Percentage of all unsuccessful nests
Abandoned Because Of:					
Observer	1	0	0	1	3.0
Flood waters	0	2	0	2	6.1
Heavy rain	1	4	0	5	15.2
Grazing animals	0	1	0	1	3.0
Brush cutting	0	1	0	1	3.0
Totals	2	8	0	10	30.3
Predation By:					
Feral cat (*Felis catus*)	1	0	2	3	9.1
Red fox (*Vulpes regalis*)	0	1	2	3	9.1
13-striped ground squirrel (*Citellus t. tridecemlineatus*)	0	1	0	1	3.0
Weasel (*Mustela* sp.)	0	1	0	1	3.0
Snake (*species undetermined*)	2	1	1	4	12.2
Crow (*Corvus b. brachyrhynchos*)	0	0	1	1	3.0
Raccoon (*Procyon l. lotor*)	0	0	1	1	3.0
Undetermined animal	2	2	1	5	15.2
Totals	5	6	8	19	57.6
Undetermined	4	0	0	4	12.1
Grand Totals	11	14	8	33	100.0

from nests that were under observation. The earliest brood observed in 1946 was believed to have been hatched about May 27; in 1947, about July 1; and in 1948, about June 1.

The average number of young hatched was 11.8 from five nests in 1946, 12.8 from four nests in 1947, and 14.8 from four nests in 1948. At eight weeks of age, the numbers had decreased to 9.2, 8.5, and 10.3 birds, respectively. Mortality was the highest during the first two weeks after hatching. For the entire study 28.6 per cent of the young birds were lost by the time they had reached the age of eight weeks.

POPULATIONS AND PRODUCTION

Data on the populations, densities, and per cent of change from previous count are given in Table 3. These records were taken during the prenesting season (March 28–April 1), the prehunting season (November 13–15), and the posthunting season (December 26–29). It is noted that on the Eldon Research Area the prenesting quail populations declined from 1946 to 1948. In 1949, however, the prenesting population was up 256 per cent over the 1948 count. This rise was accounted for through the evident shifting of coveys onto the study area during and following the 1948 hunting season (5). Records of per cent of summer gain as related to previous breeding density indicate inverse ratios of rates of gain to breeding densities (2), except for the 1948 season.

SUMMARY

An intensive investigation was made of bob-white quail nesting and production on the Eldon Research Area, a 1,600-acre tract in southeastern Iowa, from January 1946 to April 1949.

The breeding population for 1946, 1947, 1948, and 1949 was seventy-two, forty-eight, sixteen, and fifty-seven birds, respectively. Forty-six nests were found during the first three seasons by using the strip and quadrat methods. The former technique was adjudged the more successful. The nesting season covered 115 days in 1946, 135 in 1947, and 141 in 1948. Two peaks in nest commencement occurred, one in May and one in June, each season. Nonproductive lands supported more than 78 per cent of the nests and productive lands the remainder. It appeared that farm lands and margins in the cover pattern as well as suitable nesting cover influenced nest locations. The majority of nests were constructed of and located in grasses of which bluegrass predominated.

The average number of eggs per nest for 1946–1948 was slightly over fifteen. Clutch sizes decreased somewhat as the nesting season progressed.

Twenty-eight per cent of the nests hatched successfully. The heaviest nest losses occurred during June. Slightly more than 30 per cent of the nests were abandoned because of disturbance to the nests or nesting birds; climatic factors accounted for 15.2 per cent of these.

TABLE 3

DATA ON BOB-WHITE POPULATIONS—ELDON RESEARCH AREA, 1946–1949

Year	Prenesting Records (March 28–April 1)			Prehunting Records (November 13–15)			Posthunting Records (December 26–29)		
	No. of birds	Per cent change from posthunting count	Density Birds/acre	No. of birds	Per cent change from prenesting count	Density Birds/acre	No. of birds	Per cent change from prehunting count	Density Birds/acre
1946	72	....	1/22	149	107% increase	1/11	64	57% decrease	1/25
1947	48	25% decrease	1/33	133	177% increase	1/12	22	82% decrease	1/76
1948	16	37% decrease	1/100	37	131% increase	1/43	47	27% increase	1/34
1949	57	21% increase	1/28	....	....	....	....	....	....

Predators were accredited with 57.6 per cent of all the nest losses.

Data on eighteen broods revealed an average of 13.1 birds per brood at hatching. At eight weeks the average size of broods had decreased to 9.72, showing a loss of 28.6 per cent.

The prenesting quail populations on the Eldon Research Area were on a decline from the spring of 1946 to the spring of 1948.

The 1949 prenesting population on the research area increased twenty birds over the 1948 fall population. This was a 256 per cent increase over the 1948 prenesting population. The data indicate that as breeding density increased the per cent of summer gain decreased.

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THE STANNOUS CHLORIDE EQUILIBRIUM[1]

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The stabilities of the stannous chloride complexes were originally determined by Prytz[2] as follows:

$$\begin{aligned}
&Sn'' + Cl' = SnCl' \qquad && K_1 = \frac{\alpha_{SnCl'}}{\alpha_{Sn''} \times \alpha_{Cl'}} = 32 \\
&SnCl' + Cl' = SnCl_2 \qquad && K_2 = \frac{\alpha_{SnCl_2}}{\alpha_{SnCl'} \times \alpha_{Cl'}} = 5.5 \\
&SnCl_2 + Cl' = SnCl_3' \qquad && K_3 = \frac{\alpha_{SnCl_3'}}{\alpha_{SnCl_2} \times \alpha_{Cl'}} = 0.6 \\
&SnCl_3' + Cl' = SnCl_4'' \qquad && K_4 = \frac{\alpha_{SnCl_4''}}{\alpha_{SnCl_3'} \times \alpha_{Cl'}} = 0.3
\end{aligned} \qquad [1]$$

His procedure consisted of the measurement of the e.m.f. of a half cell composed of an unamalgamated tin rod in a solution containing stannous and chloride ions. From the measured e.m.f. of the half cell, its E°, and the stannous and chloride ion activities, calculated by the Debye-Huckel theory, the four constants were evaluated. This work is of questionable value because of the very probable presence of physical strains in the metal electrode and because of the use of the Debye-Huckel theory for solutions of ionic strength far above that for which the theory was originally derived and has been found to be applicable.

A later reference[3] contributed only a qualitative statement to the effect that stannous tin complexes with chloride ion.

In this paper the method of Prytz has been simplified and the inherent errors in his procedure eliminated. The four equilibrium constants appearing above are re-evaluated at an ionic strength of 2.03.

THEORY

The voltage of a concentration cell composed of two Sn, Sn″ half cells was measured. The original [Sn″] was identical in both half cells,

[1] Contribution No. 117 from the Institute for Atomic Research and Department of Chemistry, Iowa State College, Ames, Iowa. Work performed in the Ames Laboratory of the Atomic Energy Commission.

[2] Prytz, M. Komplexbildun in Stannochlorid-und Stannobromidlösung. Z. Anorg. Allgem. Chem., 172:147–166. 1925.

[3] Allison, L. R., E. J. Hartung and E. Heymann. An anomalous concentration cell. The constitution of solutions of stannous chloride in water and in hydrochloric acid. Jour. Phys. Chem., 44:1033–37. 1940.

but one half cell, II, contained varying concentrations of chloride ion. A constant ionic strength and [H′] was maintained throughout the experiment. The electrodes were tin amalgam of equal tin concentration. Thus, the system under consideration is

$$Hg\,(Sn_I),\ Sn''_I \left\{ \begin{matrix} HClO_{4I} \\ NaClO_{4I} \end{matrix} \right\} \Bigg\| \left\{ \begin{matrix} HCl_{II} \\ NaCl_{II} \\ HClO_{4II} \end{matrix} \right\} Sn''_{II},\ (Sn_I)\,Hg$$

The only source of potential is given by the Nernst equation as

$$E = 0.0128 \ln \frac{\alpha_{Sn''_{II}}}{\alpha_{Sn''_I}}, \qquad [2]$$

since the tin activities are equal in both amalgams and junction potentials are assumed eliminated by a NH_4NO_3 salt bridge.[4] Since we are here concerned only with one ionic strength, activity coefficients in [2] cancel, and, for our purposes,

$$\alpha_{Sn''} = [Sn''] \qquad [3]$$

It is seen that E° for the Sn, Sn″ half cell does not enter into consideration, thus eliminating a considerable source of error. Since perchlorate forms no complexes, $[Sn''_I] = T_{Sn}$, a concentration which can be calculated from the initial $[Sn''_I]$ and subsequent dilution. In cell II, dilution equal to that in cell I gives

$$T_{Sn_{II}} = T_{Sn_I} = T_{Sn} \qquad [4]$$

and therefore

$$Sn_{II} = T_{Sn} - \left\{ [SnCl'] + [SnCl_2] + [SnCl_3'] + [SnCl_4''] \right\} \qquad [5]$$

If we now assume that total chloride concentration, T_{Cl}, is much greater than the chloride present in the complexing species,

$$[Cl'] = T_{Cl} \qquad [6]$$

Since the stannous concentration is initially only 0.005M and decreases with increasing chloride because of dilution, this assumption is well founded. Such ionic species as $SnCl_5'''$ and $SnCl_6''''$ are not considered in this treatment because there is little evidence for their existence. Thus we may derive from [1], [5], and [6] the relation

$$[Sn''_{II}] = \frac{T_{Sn}}{T^4_{Cl}K_1K_2K_3K_4 + T^3_{Cl}K_1K_2K_3 + T^2_{Cl}K_1K_2 + T_{Cl}K_1 + 1} \qquad [7]$$

Substitution of (3), (4) and (7) in (2) gives

$$\exp\left(-\frac{E}{0.0128}\right) = \varepsilon = AT^4_{Cl} + BT^3_{Cl} + CT^2_{Cl} + DT_{Cl} + 1 \qquad [8]$$

[4] Cumming, A. C. Contributions to the studies of strong electrolytes. Trans. Faraday Society, 2: 213–21. 1907.

where

$$\begin{aligned} A &= K_1K_2K_3K_4 \\ B &= K_1K_2K_3 \\ C &= K_1K_2 \\ D &= K_1 \end{aligned} \qquad [9]$$

EXPERIMENTAL

MATERIALS

The $Sn(ClO_4)_2$, $HClO_4$, HCl, NaCl, and NH_4NO_3 were of commercial grade. $NaClO_4$ was made by the neutralization of $HClO_4$ with pure NaOH. Pure tin and triple distilled mercury were used for the amalgam.

The $Sn(ClO_4)_2$ was standardized against $Ce(ClO_4)_4$, which was previously standardized by ferrous ammonium sulfate. Any stannic tin impurities in the Sn $(ClO_4)_2$ were assumed to have a negligible effect since the stannous concentration was small.

The acids were standardized against weighed Na_2CO_3.

PROCEDURE

The acids were adjusted to equal concentration, and the $NaClO_4$ and NaCl solutions were also of equal concentration.

The amalgam was made by dissolving 1 gram of tin in 100 ml. of Hg. The α_{Sn} in the amalgam, being equal in both half cells, was immaterial for our purposes.

Equal volumes of a solution in which $[H'] = 2.00$ and $[Sn''] = 0.005$ with $\mu = 2.03$ were added to the half cells and the potential of the system measured. In every case the original potential was within 0.3 millivolts of the correct potential of zero. Then acid, salt, and water were added to the cells, perchlorates in one cell and chlorides in the other, in the appropriate quantities as determined by the desired chloride concentration and the constraints of constant [H′] and ion strength, and the potential of the cell measured. Since the concentrations of the two acids were equal, and the two salt solutions also were equal concentrations, constant [H′] and ionic strength could be maintained in both cells while [Cl′] was changed in cell II. This dilution lowered the stannous concentrations in both cells from their original values, but at any time the total volumes in the cells were equal and thus [4] was true. This procedure required that the partial molal volumes of the two acids, and likewise the two salts, be equal, and within the limits of this experiment this condition can be assumed satisfied.

A salt bridge made of 3 per cent agar solution saturated with ammonium nitrate was used. With a saturated KCl bridge a potential drift was observed, for some chloride necessarily diffuses into the body of solution. The lack of drift with the ammonium nitrate bridge allowed the elimination from consideration of stannous nitrate complexes.

An inert atmosphere of nitrogen was maintained throughout the

system to prevent air oxidation of the stannous tin. Fresh solutions of stannous perchlorate were prepared each day.

All potentiometric measurements were made with a Leeds and Northrup Student Type Potentiometer. Precision was maintained to ± 0.4 mv.

Temperature was held constant to $25.0 \pm 0.1°C$.

RESULTS

The results of this experiment are given in Table 1 and plotted in Figure 1. Figure 1 corresponds to [8]. The tolerances in Figure 1 indicate the effect of the probable error in e.m.f.

D, or K_1, is best found by taking the limiting slope at $T_{Cl} = O$ of the curve in Figure 1. With D known, three values for ε with their corresponding T_{Cl} values were taken and solution of the resulting 3rd order determinant for A, B, and C allowed us to evaluate K_2, K_3, and K_4 from [9]. The following values for the equilibrium constants were obtained:

$$
\begin{aligned}
K_1 &= 11.3 \pm 0.2 \\
K_2 &= 5.1 \pm 0.2 \\
K_3 &= 0.24 \pm 0.05 \\
K_4 &= 1.0 \pm 0.4
\end{aligned}
\qquad [10]
$$

TABLE 1

[Sn''] initial = 0.005 μ = 2.03
[H'] = 2.00   t = 25° C.

[Cl']	$-E$	$\varepsilon = \exp\left(-\frac{E}{0.0128}\right)$
0	0	1
0.010	0.0015	1.12
0.030	0.0043	1.40
0.050	0.0068	1.69
0.080	0.0103	2.23
0.100	0.0129	2.74
0.130	0.0156	3.38
0.160	0.0189	4.40
0.200	0.0222	5.72
0.250	0.0262	7.75
0.300	0.0296	10.1
0.350	0.0327	12.9
0.400	0.0356	16.1
0.450	0.0382	19.9
0.500	0.0404	24.0
0.550	0.0428	28.3
0.600	0.0451	33.8
0.650	0.0472	39.5
0.700	0.0492	46.5
0.750	0.0507	53.0
0.800	0.0528	61.3
0.850	0.0540	68.0

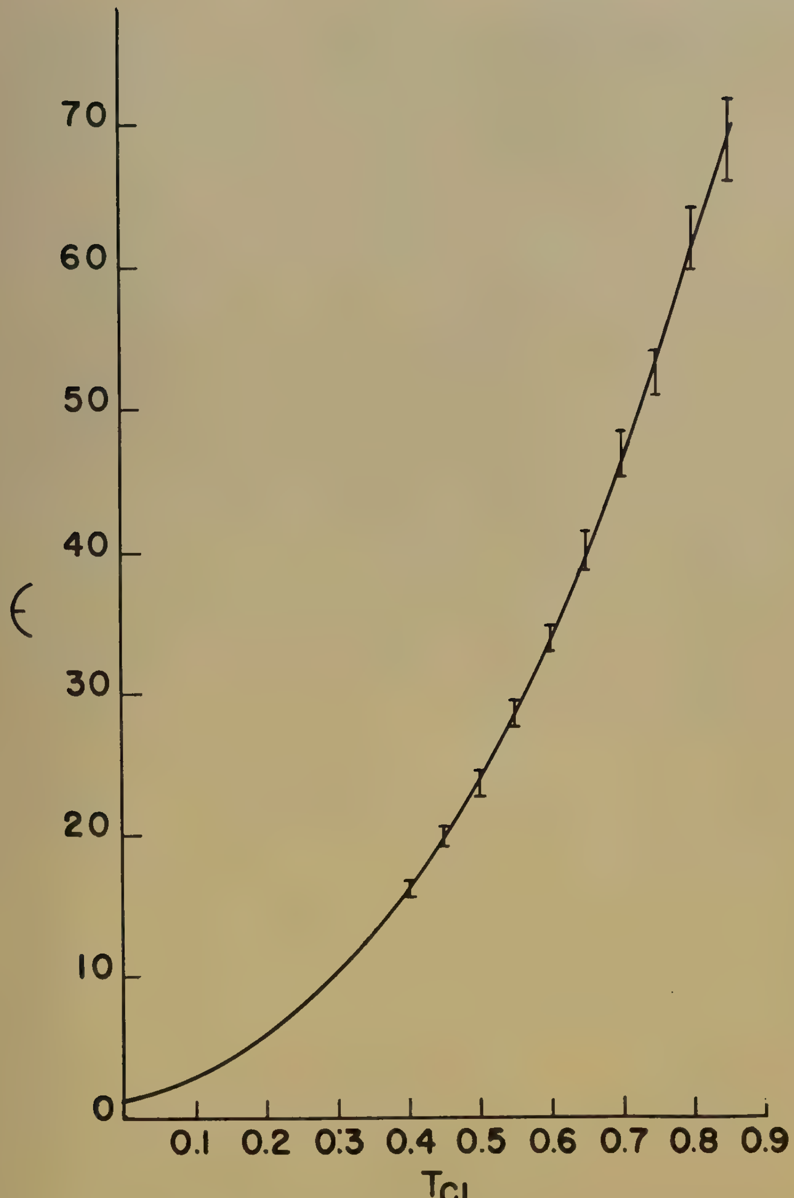

FIG. 1.—The effect of Chloride ion on ε, where $\varepsilon = \exp(-E/.0128)$.

The increasing probable error in [10] resulted from the polynomial character of T_{Cl} in [8] and the exponential nature of ε, although the precision of E was maintained throughout.

The relative proportions of species in a stannous chloride solution of $\mu = 2.03$ can be calculated by the following equations, assuming [7] is valid.

$$[Sn''] = \frac{T_{Sn}}{K_1K_2K_3K_4T^4_{Cl} + K_1K_2K_3T^3_{Cl} + K_1K_2T^2_{Cl} + K_1T_{Cl} + 1}$$

$$\begin{aligned}
[SnCl'] &= [Sn''] \cdot T_{Cl}K_1 \\
[SnCl_2] &= [Sn''] \cdot T^2_{Cl}K_1K_2 \\
[SnCl_3'] &= [Sn''] \cdot T^3_{Cl}K_1K_2K_3 \\
[SnCl_4''] &= [Sn''] \cdot T^4_{Cl}K_1K_2K_3K_4
\end{aligned}$$

and are shown in Figure 2.

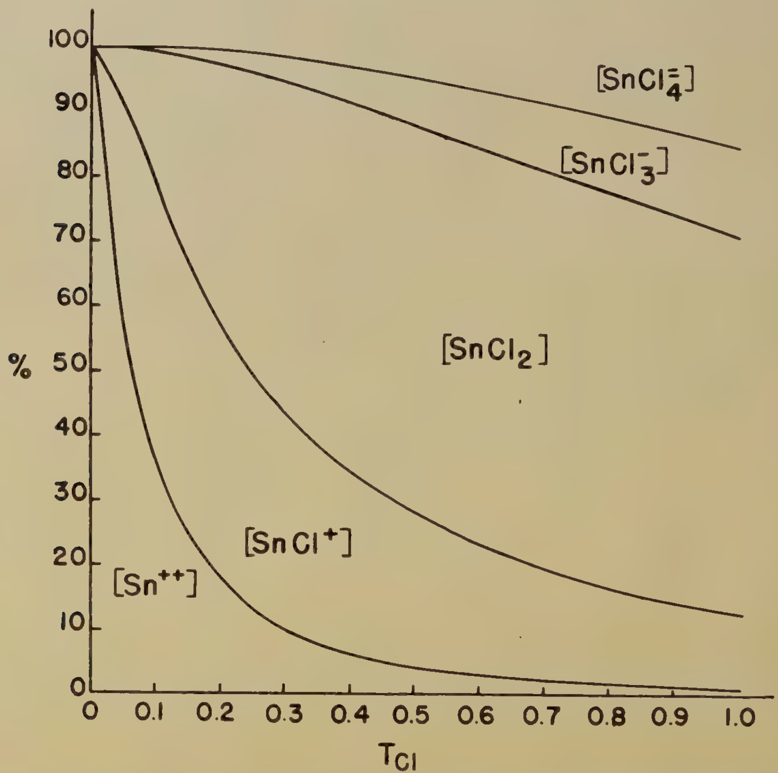

FIG. 2.—Relative magnitudes of the ionic species in a stannous chloride solution.

SUMMARY

A general method is outlined for evaluating equilibrium constants of complex ions when the parent positive ion participates in a reversible electrode reaction.

The equilibrium constants of stannous and chloride ions at an ionic strength of 2.03 were determined as follows:

$$K_1 = 11.3 \pm 0.2$$
$$K_2 = 5.1 \pm 0.2$$
$$K_3 = 0.24 \pm 0.05$$
$$K_4 = 1.0 \pm 0.04$$

FISHERIES INVESTIGATIONS ON TWO ARTIFICIAL LAKES IN SOUTHERN IOWA

III. HISTORY AND CREEL CENSUS[1]

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A limnological and fishery investigation of two eighty-acre reservoirs near Chariton, Iowa, was conducted in 1948 (1,2,3). The present paper reviews the past history of the fish populations and of their utilization. The notes on past history have been obtained largely from local residents and therefore are not complete in certain details.

HISTORY

Several species of fish have been stocked in Red Haw and East Lakes since their completion in 1935 and 1915, respectively (Tables 1 and 2). There are no reports of northern pike having been taken although a few were stocked in East Lake in 1923, 1925, and 1927. The minnows stocked in Red Haw Lake in 1939 to 1941 apparently also failed to establish themselves, as no minnows were taken in 1948. All other species which are reported to have been stocked apparently established themselves or at least survived in numbers sufficient to contribute to later catches.

A few species apparently were introduced by accident with the recorded stockings or were stocked by unauthorized persons. Green sunfish were found in both lakes, but not in numbers sufficient to affect the game fish population.

According to local residents, Red Haw was densely populated in 1937 with green sunfish and fishing for this species was opened at that time. There were reportedly thousands of these fish taken. Follow-

[1] Iowa Cooperative Fisheries Research Unit, Project No. 42 sponsored by the Industrial Science Research Institute of Iowa State College and the Iowa State Conservation Commission, with the cooperation of the U. S. Fish and Wildlife Service.

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ing this time there was a steady decline in the green sunfish population probably due to its inability to successfully compete with the other species of fish which were becoming established. During the summer

TABLE 1
NUMBERS AND SPECIES OF FISH STOCKED IN RED HAW LAKE BY THE IOWA CONSERVATION COMMISSION*

Year	Size	Large-mouth black bass	Bull-heads	Black crappie	White crappie	Bluegill	Minnows
1936	Fry	5,000	...	...	...	...	...
	Yearling	...	1,200	2,500	900	3,100	...
	Adult	...	800	...	...	300	...
1937	Fry	4,998	...	...	...	...	...
	Yearling	...	3,000	...	...	...	...
	Adult	...	2,600	...	...	...	...
1938	Fry	4,652	...	...	...	...	...
1939	Fry	4,017	...	...	...	...	...
	Yearling	1,000	1,000	...	...	...	...
	Adult	...	28,400	...	...	...	5,100
1940	Fry	300	...	12,000	200	60,000	...
	Yearling	553	...	1,100	850	...	...
	Adult	...	4,160	190	135	2,100	70,000
1941	Yearling	...	...	...	...	...	150,000
	Adult	...	4,000	...	...	...	...
1942	Fry	5,633	...	...	7,500	...	...
	Adult	...	...	...	60	...	...
1943	Fry	1,800	...	...	42,294	13,300	...
1944	Fry	6,047	...	...	...	7,985	...
	Adult	...	4,000	...	...	...	...
1945	Fry	1,504	...	11,880	...	39,300	...
1946	Fry	4,047	...	...	65	...	...
	Adult	...	3,000	...	...	...	...
1947	Fry	8,882	...	11,323	...	...	...
	Yearling	...	2,000	...	...	...	...
	Adult	...	4,000	...	...	...	...

* Data provided by E. B. Speaker, superintendent of the Biology Section.

of 1948 only three or four green sunfish were taken despite intensive sampling by several methods.

Yellow perch were stocked in East Lake but apparently not in Red Haw. In both lakes, yellow perch are now abundant but do not attain a large size. Practically all of the perch taken in 1948 were under seven inches in total length. On account of their abundance, some of these

small perch are taken by fishermen. Warmouth, not reported to have been stocked, are quite common in Red Haw but rare in East Lake.

Bullheads were reported to have been very abundant in Red Haw when the lake opened to general fishing in 1939. Fishing was good for a while but gradually tapered off. In 1948 bullhead fishing was too slow to satisfy most fishermen. Bullhead fishing is reported to have varied in East Lake with better fishing after each stocking.

FIG. 1.—A six and three-quarter pound largemouth from Red Haw Lake.

Largemouth black bass fishing with plugs is said to have been excellent in Red Haw from shortly after the lake was opened to fishing until about 1943. In 1948 the dominant size group was from three-fourths of a pound to a pound and one-quarter. Plug fishing was rather poor, but fly fishing with the proper bait was good. If sufficient numbers of this dominant size group survive, plug fishing should improve and during the summer of 1950 it should be excellent. In East Lake largemouth black bass fishing has been fairly good since bass first reached catchable size following the first stocking. During the summer of 1948 crappie fishermen, using minnows, took quite a few three-quarter to one and one-quarter pound bass. Fly fishing was excellent throughout most of the summer but plug fishing was poor.

Bluegill fishing, as far as could be determined, has been good in both lakes since fishing opened. This species now supports most of the

TABLE 2

NUMBER AND SPECIES OF FISH STOCKED IN EAST LAKE BY THE IOWA CONSERVATION COMMISSION *

Year	Size	Largemouth black bass	Bullheads	Channel catfish	Black crappie	White crappie	Yellow perch	Yellow pike-perch	Pike	Bluegill
1922	Fry							225,000		
1923	Fry	1,700			1,000			225,000		4,500
	Yearling			750					50	
	Adult					50				
1924	Fry							100,000		
1925	Fry	200							250	
	Yearling			750	500					1,000
1926	Fry				2,050			450,000		400
	Yearling			50						
1927	Fry	385			2,700			150,000	50	3,000
	Yearling			650			75			
1929	Fry			210			400	200,000		350
1930	Fry							350		
1931	Fry						4,500			5,500
	Adult		20,000							
1932	Fry							200,000		4,800
	Yearling	65		600	300					
	Adult		5,000							

TABLE 2 (Continued)

Year	Size	Largemouth black bass	Bullheads	Channel catfish	Black crappie	White crappie	Yellow perch	Yellow pike-perch	Pike	Bluegill
1937	Fry	...	...	...	1,000	...	...	...	...	2,000
	Adult	...	...	...	500	...	...	...	...	600
1938	Fry	4,000	...	...	...	...	...	...	...	...
	Adult	65	...	...	...	...	...	...	...	...
1939	Yearling	4,017	36,800	...	...	...	...	...	...	...
	Adult	...	1,000	...	...	...	...	...	...	...
1940	Fry	...	...	...	6,000	100	2,725	...	...	20,000
	Yearling	...	...	1,172	75	...	...	...	...	...
	Adult	...	125	...	10	...	...	...	...	50
1942	Fry	5,800	...	...	...	...	...	...	...	...
1943	Fry	1,000	...	...	...	...	...	...	...	...
1944	Fry	6,405	...	...	...	...	...	...	...	11,955
1946	Fry	...	...	...	1,500	...	...	...	...	...
	Adult	...	...	...	35	...	...	...	...	...
1947	Fry	3,500	...	...	2,600	...	...	...	...	...
	Adult	...	...	...	310	...	...	...	...	...

* Data from E. B. Speaker, superintendent of the Biology Section. No fish stocked in 1928, 1933–36, 1941, 1945. In 1915, 80,000 miscellaneous fish were stocked and 40,000 minnows were stocked in 1942.

middle and late summer fishing. Specimens up to a pound were taken from Red Haw during the summer of 1948, but the average weight was approximately one-half pound. During the summer of 1948 there were thousands of quarter-pound bluegills present in East Lake. In fact, few specimens of any other size were caught. This uniformity of size is

FIG. 2.—Four-hour catch of bluegill from Red Haw Lake. Fish ranged in weight from one-half to one pound.

said to have developed during the past seven or eight years. In addition to being present in great numbers, these quarter-pound bluegills bit readily but were not harvested in appreciable numbers.

At the time of the field investigations Red Haw had a good population of large black crappie and some white crappie. These two species supported most of the fishing early in the season. During the summer

of 1948, specimens weighing as much as two pounds were caught, and many one-pound individuals were examined. White crappie were found to be abundant in East Lake. Crappie fishing is reported to have been continually good at least during the spring. In April and June, 1948, phenomenal numbers of one-quarter-pound crappies were taken in short periods of fishing. Most of these crappies and those caught in experimental traps were white crappies. Black crappie, although they have been heavily stocked, are scarce compared to the whites.

Of particular interest are results of the substantial stockings of channel catfish and yellow pikeperch in East Lake. For several years

FIG. 3.—An eighteen and a half-pound channel catfish from East Lake.

after the latter species was stocked, there were fairly good catches of two- and three-pound fish. In the 1920's, during a flood period, many yellow pikeperch were lost over the spillway and captured. Some of these specimens weighed up to six pounds. Following this flood, practically no more of this species were taken. Intensive sampling in the course of the present study failed to produce a single pikeperch.

In 1935 channel catfish fishing in East Lake became good. For a couple of weeks two to ten channel catfish were taken each day by twenty-five to thirty fishermen. These fish were mostly 1- to 4.5-pound specimens. Since 1935 the number caught has steadily decreased, although the sizes of the fish have steadily increased. In 1939 a twenty-

one-pound specimen was taken. During this past summer fifty channel catfish ranging in weight from 2 to 18.5 pounds were recorded. There was no indication that these fish reproduced in this lake.

In October of 1948 a fifty-four-pound shovelhead catfish was taken in East Lake. A catfish of similar size was also caught during the spring of 1949. The latter specimen was not examined by the writer but descriptions indicate that it also was a shovelhead. It is interesting to note that this species of catfish grew quite successfully in the lake. In July, one channel catfish was found floating dead in Red Haw. This specimen was twenty-seven inches in total length. Gill netting, trapping, seining, and angling failed to produce any other fish of this species.

THE 1948 CREEL CENSUS

The creel census of Red Haw is estimated to represent 50 per cent of each day's boat fishing. Bank fishing was important early in the season when five to ten bullhead fishermen bank-fished each evening. Although it was not possible to conduct a successful creel census on the night fishing, enough information was gathered to show that in two to three hours of fishing a party of three or four persons would catch two or three bullheads, many of which weighed more than a pound. After two or three weeks, this bank fishing became insignificant.

For all other types of fishing the daily record included number of persons, hours fished by each, catch for each, and the type of gear used. This information was condensed by two-week intervals (Tables 3, 4, 5, and 6).

Plug fishing in Red Haw during the 1948 season was poor (Table 3). On an average it required twenty hours of fishing to produce a legal bass. It appeared that the most likely explanation for the poor plug fishing was the predominance of three-quarter-pound bass which were too small to be good "strikers." The fact that fly fishing for bass

TABLE 3
Plug Fishing on Red Haw Lake, 1948

Dates	Man Hours	No. of Bass	Catch/Hour
6/1 – 6/14	158	10	.063
6/15– 6/28	107	7	.065
6/29– 7/12	128	3	.023
7/13– 7/26	72	0	.000
7/27– 8/ 9	15	0	.000
8/10– 8/23	35	2	.057
8/24– 9/ 6	42	7	.167
9/7 – 9/20	50	1	.020
9/21–10/ 4	20	1	.050
10/5 –10/18	0	0	....
10/19–11/ 1	40	2	.050
11/2 –11/16	5	0	.000
Totals and mean	672	33	.049

was good supports the thesis that size, and not abundance, was the important factor. The fly rod lures are, of course, smaller and without doubt more suitable for attracting the small bass. The majority of bass taken by plug fishing weighed from three-quarters to one and one-quarter pounds. The largest recorded was six and three-quarter pounds.

Minnow fishing can be considered primarily crappie fishing, but bass, bluegill, and perch were also caught (Table 4). The fishing success for crappie from May 15 through June 15 was high enough to satisfy most fishermen. The actual catch in numbers was not exceptional, but the fish caught at this time averaged around a pound. The poor success later in the season is more or less characteristic of crappie fishing. Just what causes the success in fishing to change so abruptly is not understood. In East Lake it appeared to be due to a change in distribution and a general reduction in the schooling tendency. It was not possible to find similar evidence in Red Haw, although such a situation may have occurred.

Some of the fishermen used a variety of baits on the same trip, and therefore their catches cannot be included with other data. The fishing classed under mixed baits (Table 5) can almost be considered worm fishing, for worms constituted the greater part of the various baits. Bluegills, as would be expected, made up the greater part of the mixed bait catch. As far as this species was concerned, mixed bait fishing produced a high degree of success. The fishing success could have been greatly increased had the average fisherman been better informed as to the best equipment and techniques to catch this species.

It will be noted the higher bluegill catch both by mixed baits (Table 5) and by worms (Table 6) started later and continued longer than the catch for the other species. Early in the spring the majority of the fishermen and perhaps the best ones devoted most of their time to crappie fishing. Also at this time the bluegills moved in toward the banks to spawn, and were not concentrated in the deeper water where they were more vulnerable to the tactics used by most worm fishermen. As the season progressed and the bluegills, particularly the females, became concentrated in the deep water, one could rely on them to bite even when other fishing was poor.

With the exception of that by two or three individual fishermen, no fly fishing was done on the two lakes during the period of study. The one or two individuals who did use fly rods had good success taking bass and bluegill. Fly fishing was also used with success to collect bass and bluegill for study purposes.

Considering all four types of fishing there were 3,972 fish recorded. The part contributed by each species was: bluegill 3,029, crappie 522, bass 249, perch 156, warmouth 4, green sunfish 8, and bullheads 4. It is apparent from this that the bluegill was the most important species.

Since the amount of help available was not sufficient to conduct a full-time creel census on both lakes, the data for East Lake are quite

TABLE 4

FISHING WITH MINNOWS, RED HAW, 1948

Dates	Man Hours	Crappie		Bass		Bluegill		Perch		Misc.*
		No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	
5/15– 5/31	456	139	.305	16	.035	5	.011	12	.026	
6/1 – 6/14	570	129	.226	24	.042	111	.195	22	.038	4
6/15– 6/28	260	23	.088	8	.031	36	.138	2	.077	1
6/29– 7/12	344	48	.140	16	.046	53	.154	4	.012	
7/13– 7/26	54	1	.018	0	.000	1	.018	0	.000	
7/27– 8/9	9	0	.000	0	.000	8	.889	0	.000	
8/10– 8/23	128	1	.078	4	.031	3	.234	1	.078	
8/24– 9/6	36	0	.000	0	.000	0	.000	0	.000	
9/7 – 9/20	123	5	.041	3	.024	6	.488	0	.000	
9/21–10/4	13	0	.000	0	.000	2	.154	3	.231	1
10/5 –10/18	24	0	.000	0	.000	1	.417	1	.417	
10/19–11/1	12	0	.000	0	.000	0	.000	0	.000	
11/2 –11/16	2	0	.000	0	.000	0	.000	0	.000	
Totals and Means	2,031	346	.170	71	.035	226	.111	45	.022	6

* Three green sunfish, two warmouths, one bullhead.

TABLE 5

Mixed Bait Fishing, Red Haw, 1948

Dates	Man Hours	Crappie		Bass		Bluegill		Perch		Misc.*
		No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	
5/16– 5/31	44	0	.000	0	.000	0	.000	8	.182	
6/1 – 6/14	235	24	.102	12	.051	56	.238	27	.115	3
6/15– 6/28	406	16	.039	29	.071	309	.761	5	.012	
6/29– 7/12	300	17	.057	12	.040	346	1.153	7	.023	
7/13– 7/26	458	10	.022	8	.017	293	.640	2	.004	
7/27– 8/9	530	19	.036	8	.015	384	.724	5	.009	1
8/10– 8/23	493	39	.079	23	.047	159	.322	9	.018	1
8/24– 9/6	213	5	.023	13	.061	37	.174	3	.014	1
9/7 – 9/20	286	2	.007	10	.035	42	.147	9	.031	
9/21–10/4	131	6	.046	12	.092	25	.191	2	.015	
10/5 –10/18	52	0	.000	6	.115	3	.058	5	.096	
10/19–11/1	28	0	.000	2	.071	0	.000	0	.000	
11/2 –11/16	7	0	.000	0	.000	1	.143	0	.000	
Totals and Means	3,183	138	.043	135	.042	1,655	.520	82	.002	6

* Three green sunfish, two warmouths, one bullhead.

TABLE 6

Worm Fishing, Red Haw, 1948

Dates	Man Hours	Crappie		Bass		Bluegill		Perch		Misc.*
		No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	
6/1 – 6/14	29	0	.000	0	.000	42	.690	0	.000	
6/15– 6/28	205	3	.146	2	.098	166	.810	0	.000	
6/29– 7/12	396	26	.066	5	.013	510	1.290	19	.048	
7/13– 7/26	214	3	.014	1	.005	161	.752	1	.005	4
7/27– 8/9	154	2	.013	1	.006	175	1.140	2	.013	
8/10– 8/23	81	1	.012	0	.000	49	.605	0	.000	
8/24– 9/6	98	0	.000	0	.000	3	.031	4	.041	
9/7 – 9/20	58	3	.052	0	.000	15	.258	1	.017	
9/21–10/4	42	0	.000	1	.024	21	.500	2	.048	
10/5 –10/18	4	0	.000	0	.000	6	1.500	0	.000	
10/19–11/1	10	0	.000	0	.000	0	.000	0	.000	
11/2 –11/16	4	0	.000	0	.000	0	.000	0	.000	
11/17–11/21	4	0	.000	0	.000	0	.000	0	.000	
Totals and Means	1,299	38	.029	10	.008	1,148	.884	29	.022	4

* Two green sunfish, 2 bullheads.

incomplete, and it is not possible to estimate the percentage of the fishing represented. As far as the data goes, it is believed to correctly represent the fishing conditions.

Sampling indicated the bass of East Lake to be just as abundant and just as large as those of Red Haw, and yet there was very little plug fishing done on this lake (Table 7).

For about a month crappie were biting exceptionally well along the East Lake dam. During this period minnow fishing was heavy and fishing success for crappies was high. Fair catches of bass, perch, and bluegill were also made (Table 8).

Mixed bait fishing (Table 9) showed very poor success for all species. It was noted that on Red Haw bait fishing could almost be considered to be worm fishing. On East Lake, however, the situation was quite different. Most of the baits used were cheese bait, liver, and

TABLE 7
EAST LAKE, PLUG FISHING, 1948

Dates	Man Hours	Number of Bass	Catch per Hour
6/1 –6/14	9.5	3	.316
6/15–6/28	10.0	0	.000
6/29–7/12	24.0	1	.042
Totals and Means	43.5	4	.092

other materials considered attractive to channel catfish. The majority of these baits and the manner in which they were used practically excluded the catching of any species other than the catfishes (Table 9).

Worm fishing in East Lake (Table 10) produced the highest fishing success recorded. The catch was not only high but remained so throughout the summer. Bluegills made up the majority of this catch.

By all types of fishing, 834 fish were recorded caught in East Lake. The part contributed by each kind of fish was as follows: crappie 407, bluegill 307, perch 71, bass 40, and channel catfish 9. The crappie thus appear to have supported most of the East Lake fishing. Not many channel catfish were caught, but they were a real attraction to many fishermen. There were approximately 100 catfish caught during the summer. They ranged in size from two to eighteen pounds with the majority of them from six to ten pounds. Among the boat-owning fishermen there was more interest shown in trolling for channel catfish than for any other type of fishing.

SUMMARY

Red Haw and East Lake are two artificial lakes located in southern Iowa. Of the various species of fishes stocked in the two lakes the largemouth black bass, bluegill, and yellow perch have been notably successful. The white crappie has been successful in East Lake whereas

TABLE 8

MINNOW FISHING, EAST LAKE, 1948

Dates	Man Hours	Crappie		Bass		Bluegill		Perch		Misc.*
		No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	
5/15–5/31	311.5	309	.992	8	.026	54	.173	42	.135	1
6/1 –6/14	74.0	33	.446	4	.054	12	.162	0	.000	
6/15–6/28	24.0	7	.286	1	.041	5	.204	0	.000	
6/29–7/12	14.0	0	.000	0	.000	5	.357	10	.714	
7/13–7/26	9.0	1	.111	0	.000	1	.111	3	.333	
7/27–8/9	56.5	16	.283	7	.124	2	.035	4	.071	
8/10–8/23	2.5	0	.000	0	.000	0	.000	0	.000	
Totals and Means	491.5	366	.744	20	.041	79	.161	57	.116	1

* One warmouth.

TABLE 9

MISCELLANEOUS AND MIXED BAITS, EAST LAKE, 1948

Dates	Man Hours	Crappie		Bass		Bluegill		Catfish		Misc.*
		No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	
5/15–5/31	2.0	2	1.000	0	.000	5	2.500	0	.000	5
6/1 –6/14	34.5	19	.550	0	.000	18	.522	0	.000	3
6/15–6/28	25.5	0	.000	0	.000	3	.118	0	.000	
6/29–7/12	66.0	1	.015	7	.106	7	.106	0	.000	
7/13–7/26	25.5	0	.000	0	.000	0	.000	0	.000	
7/27–8/9	95.5	7	.073	7	.073	7	.073	5	.052	
8/10–8/23	28.5	8	.281	2	.070	32	1.123	2	.070	
8/24–9/6	14.0	0	.000	0	.000	0	.000	0	.000	
9/7 –9/20	1.5	0	.000	0	.000	0	.000	0	.000	
Totals and Means	293.0	37	.126	16	.055	72	.246	7	.024	8

* Eight yellow per ch.

the black has been the more successful in Red Haw. Golden shiner and channel catfish are well established in East Lake but scarce in Red Haw. Several substantial stockings of bullheads in both lakes failed to sustain good fishing. At one time green sunfish were very abundant in Red Haw but this species was rare in the lake during the summer of 1948. Yellow pikeperch are reported to have survived and to have supported fishing for a few years following the stockings.

TABLE 10
WORM FISHING, EAST LAKE, 1949

Dates	Man Hours	Crappie		Bluegill		Perch		Misc.*
		No.	Catch/hr.	No.	Catch/hr.	No.	Catch/hr.	
5/15–5/31......	15.0	2	.133	25	1.667	1	.066	
6/1 –6/14......	....	....	....	......	....	....	....	....
6/15–6/28......	50.0	2	.040	31	.620	0	.000	1
6/29–7/12......	49.5	0	.000	22	.444	5	.101	
7/13–7/26......	11.5	0	.000	6	.522	0	.000	
7/27–8/9	12.5	0	.000	10	.800	0	.000	
8/10–8/23......	12.0	0	.000	25	1.562	0	.000	
8/24–9/6	21.0	0	.000	37	1.762	0	.000	
Totals and Means.......	175.5	4	.023	156	.889	6	.034	1

* One channel catfish.

The creel census data for Red Haw Lake showed the bluegill to be first in importance and the black crappie second. The largemouth black bass catch for fishermen using plugs was relatively poor. The East Lake data placed the white crappie first and the bluegill second in importance. The largemouth black bass and channel catfish catches were poor, but these species rated high from the standpoint of recreation provided.

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ELECTROPHORETIC STUDIES ON SWINE

I. COMPOSITION AND VARIABILITY OF THE PLASMA OF THE NORMAL ADULT FEMALE[1]

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Investigations of the relationship of baby pig livability to the nutritional plane of the sows during gestation and lactation have indicated that the problem is not entirely one of nutrition. As a supplement to the nutritional experiments, electrophoretic studies of serum and plasma of the sows and their litters and of the sows' whey were instigated.

Electrophoretic analyses of individual and pooled samples of swine serum and plasma have been reported. Koenig and Hogness (3) studied pooled samples from large numbers of slaughter hogs. Deutsch and Goodloe (1), Moore (4), and Svensson (6) examined individual samples. In each case information was lacking concerning the previous history, state of health, and nutritional level of the animals selected for experimentation.

Equally as important as knowledge of the normal average composition is knowledge of the variations in composition encountered in normal, healthy individuals. Such data is very limited, to date, for any species. Moore and Lynn (5) have reported a statistical analysis of the results of twelve of their own measurements plus thirteen measurements of other workers on normal human plasma. While such studies cover supposedly healthly individuals on adequate diets, the diets certainly differ and are not readily controllable as in the case of animals.

In this report an attempt has been made to determine the normal electrophoretic composition and the individual variability of the plasma of young disease-free female swine on an adequate, controlled nutritional plane.

EXPERIMENTAL

All gilts used in these experiments were used previously in growing-fattening experiments in which excellent rations were fed, as reflected in their daily gains and efficiency of feed utilization. All were at least second generation dry-lot raised and those in a given experiment were either sisters or half sisters.

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[1] Journal paper No. J-1736 of the Iowa Agricultural Experiment Station, Ames, Iowa; Project 959.

EXPERIMENT 1

Twenty gilts were selected at random from thirty-four which had been for two months on a ration consisting of ground yellow corn, soybean oil meal, dehydrated alfalfa meal, mineral mix and irradiated yeast. This basal ration was reinforced variously with meat scraps, fish meal, or fish solubles.

Twenty ml. of blood was collected from an ear vein using a hypodermic syringe and twenty-two gauge needle. To prevent clotting, the syringe was rinsed in a saturated solution of sodium oxalate and the sample expelled into a tube containing a sufficient amount of sodium oxalate to give a 1.5 per cent solution in a 20 ml. sample. The samples were immediately chilled in ice water, the plasma separated by centrifugation within a few hours, and preserved by freezing.

Upon thawing the samples, preparatory to dilution for electrophoresis, it was found that fibrin clots had formed in most instances. The clots were removed and the serum diluted 1:4 with a phosphate buffer composed of 0.0184 $M.K_2HPO_4$, 0.0020 $M.KH_2PO_4$, and 0.1280 M NaCl. The ionic strength was 0.184 and the pH 7.6. Dialysis of the 25 ml. samples was for a period of twenty-four hours at 2°C. through Visking sausage casing, with agitation, against two liters of the buffer.

EXPERIMENT 2

In order to check the reproducibility of the technique two samples of blood were collected from a sow using the following technique. It was found that by placing a tight rubber band at the base of the ear, venous pressure was increased sufficiently to cause protrusion of the superficial ear veins. An eighteen-gauge needle was easily introduced into a vein and blood collected directly and rapidly into a chilled saturated solution of sodium oxalate (3.5 per cent).[2] Preservation was by lyophilization rather than freezing, and no clotting was encountered. For analysis the dry samples were redissolved in buffer to give solutions slightly over 1 per cent in concentration. Following dialysis the solutions were diluted with dialyzate to give a refractive increment of 0.0020 between solution and buffer. A series of forty micro-Kjeldahl determinations on such solutions have shown this increment to correspond to a concentration of 9.85 ± 0.11 mg. of protein per ml. (using the conversion factor 6.25). The results of two runs on one sample and three on the other are summarized in Table 2.

EXPERIMENT 3

The swine in this experiment consisted of two groups of four gilts each. The eight had all been on different diets at the time of the first bleeding. Gilts 1 and 5 received corn plus 35 per cent protein supple-

[2] This anticoagulant was selected after exploration of many combinations, and found to be very satisfactory. Hemolysis was found to occur quite regularly with various dilutions of citrate as well as with solid oxalate.

TABLE 1

ELECTROPHORETIC ANALYSES OF SEVENTEEN SWINE PLASMAS (EXP. I) (CALCULATED ON A FIBRINOGEN–FREE BASIS)

	Albumin			Alpha globulin			Beta globulin			Gamma globulin		
	Desc.	Asc.	Av.	Desc.	Asc.	Av.	Desc.	Asc.	Av.	Desc.	Asc.	Av.
	35.3	42.3	38.8	19.0	18.2	18.6	12.4	12.2	12.3	33.3	27.3	30.3
	44.5	42.6	43.6	21.0	13.4	16.7	11.0	15.5	13.2	23.5	28.5	26.0
	43.7	40.5	42.1	15.4	21.7	18.0	14.3	16.9	15.6	26.6	20.9	23.7
	51.6	45.1	48.3	17.9	19.1	18.5	12.9	11.3	12.1	17.6	24.5	21.0
	43.7	33.8	38.8	16.9	15.9	16.4	13.4	17.6	15.5	26.0	32.7	29.4
	46.4	43.7	45.0	20.3	18.0	19.1	11.7	10.1	10.9	21.6	28.2	24.9
	44.5	41.5	43.5	19.8	19.0	19.4	11.4	12.1	11.8	24.3	27.4	25.8
	45.2	36.6	40.9	19.4	18.2	18.7	11.9	13.2	12.6	23.5	32.0	27.7
	48.9	44.7	46.8	15.2	14.2	14.7	15.2	14.5	14.8	20.7	26.6	23.7
	38.5	47.2	42.8	20.3	17.6	19.0	12.8	13.6	13.2	28.4	21.6	25.0
	44.6	42.8	43.7	19.1	18.4	18.7	12.4	13.0	12.7	23.9	25.8	24.8
	42.2	43.7	42.9	19.7	16.5	18.1	11.7	10.6	11.2	26.4	29.2	27.8
	38.2	40.4	39.3	22.3	17.9	20.1	12.4	12.7	12.6	27.1	28.0	27.6
	45.0	45.5	45.2	20.9	19.0	19.7	15.8	15.5	15.6	18.8	20.0	19.4
	46.8	47.5	47.1	14.9	19.7	17.3	17.3	15.5	16.4	21.0	17.3	18.5
	53.2	43.3	48.3	20.1	21.9	21.0	8.1	10.0	9.0	18.6	24.8	21.7
	53.6	57.7	55.6	21.4	20.9	20.2	15.6	11.5	13.6	9.4	9.9	9.6
Average	45.0	43.5	44.3	19.0	18.2	18.5	13.0	13.3	13.1	23.0	25.0	23.9
C. V.	10.0	9.2	8.8	11.4	6.5	11.2	15.8	17.8	14.5	21.3	21.1	18.5

TABLE 2

RESULTS OF FIVE ELECTROPHORETIC ANALYSES IN A SINGLE INDIVIDUAL

Sample	Albumin			Alpha globulin			Beta globulin			Fibrinogen			Gamma globulin		
	Desc.	Asc.	Av.	Desc.	Asc.	Av.	Desc.	Asc.	Av.	Desc.	Asc.	Av.	Desc.	Asc.	Av.
I	36.6	39.3	37.9	19.9	15.4	17.7	10.3	10.9	10.6	14.4	14.2	14.3	18.8	20.2	19.5
I	37.4	36.7	37.1	21.0	17.7	19.3	9.9	11.1	10.5	13.7	15.9	14.8	18.0	18.6	18.3
I	37.7	37.9	37.8	21.4	17.6	19.5	10.7	10.6	10.6	13.9	15.9	14.9	16.3	18.0	17.2
II	39.2	36.0	37.6	20.0	16.9	18.4	10.8	10.8	10.8	12.4	14.7	13.6	17.6	21.6	19.6
II	37.8	35.9	36.9	21.2	17.0	19.1	12.0	10.3	11.2	12.4	15.7	14.0	16.6	21.1	18.8
Average	37.8	36.4	37.5	20.7	16.9	18.8	10.7	10.7	10.7	13.4	15.3	14.3	17.5	19.9	18.7
C. V.	2.6	4.6	1.2	3.4	10.9	3.9	7.4	2.9	2.6	6.9	5.2	3.8	5.9	7.8	5.3

TABLE 3

ELECTROPHORETIC ANALYSES OF SIXTEEN SWINE PLASMA. SAMPLES FROM EIGHT INDIVIDUALS COLLECTED AT THE BEGINNING AND TERMINATION OF A TWO MONTH TIME INTERVALL

Sow No.	Albumin			Alpha globulin			Beta globulin			Fibrinogen			Gamma globulin			Total protein mg/ml
	Desc.	Asc.	Av.	Desc.	Asc.	Av.	Desc.	Asc.	Av.	Desc.	Asc.	Av.	Desc.	Asc.	Av.	
1	40.0	45.5	44.3	17.1	16.9	17.0	10.9	10.1	10.5	10.6	9.8	10.2	17.4	17.7	17.5	82.7
2	46.8	45.9	46.3	17.2	17.6	17.4	11.9	11.4	11.7	8.5	9.2	8.9	15.6	15.8	15.7	58.7
3	39.8	40.2	40.0	17.7	18.8	18.3	13.9	8.7	11.3	12.4	14.8	13.6	16.2	17.4	16.8	51.2
4	40.6	39.9	40.2	17.4	17.5	17.5	13.0	11.0	12.0	11.6	12.2	11.9	17.4	19.4	18.4	70.3
5	41.6	39.3	40.5	18.3	17.2	17.8	11.3	11.2	11.2	12.0	12.3	12.1	16.9	20.0	18.4	56.1
6	40.3	42.3	41.3	17.7	18.3	18.0	12.9	11.3	12.1	10.8	9.5	10.1	18.4	18.6	18.5	60.1
7	38.9	39.5	39.2	19.3	21.5	20.6	14.1	11.6	12.8	10.4	12.0	11.2	16.9	15.5	16.2	85.3
8	40.4	39.8	40.1	17.8	17.0	17.4	14.0	10.7	12.4	10.2	12.1	11.2	17.5	20.4	18.9	74.0
1*	42.4	41.0	41.7	16.5	16.4	16.5	12.0	10.6	11.3	9.6	9.4	9.5	19.5	22.6	21.0	84.9
2*	40.8	44.3	42.5	19.3	18.0	18.7	10.6	9.8	10.2	10.0	9.5	9.8	19.3	18.3	18.8	74.9
3	40.8	37.8	39.3	16.5	16.4	16.5	13.0	12.2	12.6	10.4	10.7	10.6	19.1	22.9	21.0	83.1
4*	41.8	39.2	40.5	18.0	17.9	18.0	11.3	11.4	11.3	10.6	9.9	10.3	18.3	21.6	19.9	63.3
5	40.8	40.6	40.7	18.3	18.3	18.3	13.8	12.2	13.0	11.9	11.4	11.7	15.1	17.5	16.3	79.1
6	38.2	42.5	40.3	20.7	19.3	20.0	10.4	10.7	10.6	11.4	11.1	11.3	19.3	16.4	17.8	88.7
7*	41.7	39.3	40.5	20.0	20.9	20.5	11.0	11.5	11.3	10.7	11.9	11.3	16.5	16.4	16.4	76.5
8*	34.9	33.2	34.1	20.7	21.9	21.3	11.2	11.3	11.3	13.2	14.9	14.0	20.1	18.6	19.3	81.3
Average	40.6	40.6	40.7	18.3	18.4	18.4	12.2	11.0	11.6	10.9	11.3	11.1	17.7	18.7	18.2	67.4
C. V.	6.1	8.4	7.0	7.6	9.4	8.4	10.9	8.1	7.8	9.6	15.8	15.6	8.6	12.3	9.1	19.0

* These gilts had been bred from 8 to 17 days preceding the second bleeding.

ment, a very complete ration containing both animal and plant protein and minerals. Gilt 3 received a basal ration of corn, soybean meal, minerals, and vitamins A and D. The others received this diet plus 5 per cent alfalfa meal plus the following: 0.5 per cent vitamin B_{12} (Gilt 2), 6 per cent distillers' solubles (Gilt 4), 2 per cent distillers' solubles and 1 per cent condensed fish solubles (Gilt 6), 2 per cent condensed fish solubles (Gilt 7) and 0.2 per cent A.P.F. (Gilt 8). Following the first bleeding gilts 1 through 4 were placed on a basal ration of corn, expeller soybean oil meal, ground limestone, steamed bone meal, and iodized salt. Gilts 5 through 8 received this basal ration plus dehydrated alfalfa meal, animal protein, trace minerals and a butyl fermentation product, B–X500 (a source of B complex vitamins). A period of two months elapsed before the second bleeding. Bleeding, preservation of samples, and analysis were carried out as in Experiment 2.

ELECTROPHORETIC TECHNIQUE

Electrophoretic analyses were carried out in a double-length, single-section cell of the Tiselius type. Resolution of the boundaries was accomplished by means of a Philpot-Svensson type optical system employing a 35 mm. camera as previously described by one of us (6). All patterns were enlarged approximately three diameters over the original cell dimensions and traced on paper. Where resolution was incomplete, perpendiculars were dropped from the minimal point between components to the base line. Areas were measured by planimetry. All analyses reported herein were carried out in the phosphate buffer previously mentioned. The relatively high ionic strength and consequent low potential gradient necessitate rather long electrophoresis periods, approximately five hours, for optimal resolution. This was deemed justifiable in view of the improved enantiography of the patterns.

RESULTS AND DISCUSSION

In Figure 1 is reproduced an electrophoretic pattern typical of those obtained in these studies. It will be noted that resolution between the two α- and the two β-globulins is not attained in this buffer. This is in accordance with the findings of Koenig and Hogness (3). On the other hand, as pointed out by these workers, resolution of the gamma component is better in this buffer than in veronal, hence its use in these studies.

In Table 1 are summarized the electrophoretic analyses of seventeen of the twenty plasma samples from the gilts in Experiment 1. (Runs on three of the samples were unsatisfactory because of excessive hemolysis.) In most instances little or no fibrinogen was observed in the patterns, a consequence of the clotting which occurred upon thawing the samples. The results are therefore calculated on a fibrinogen-free basis. The degree of variability in these results, as in those presented in the other tables, is indicated by inclusion of coefficients of variation. The variations are of the same order of magnitude, possibly somewhat

less, than those observed by Moore and Lynn (5) in the case of human plasmas.

It was felt at the time these results were obtained that lack of precision in the electrophoretic analyses was responsible in part for these variations. In particular it was felt that better control of the concentration through adjustment of the solution to a definite refractive increment (thus providing constant total pattern area) might improve this situation. Such control was hence used in all subsequent analyses. Duplicability of repeated measurements using the improved technique is indicated by the extremely low coefficients of variation in Table 2.

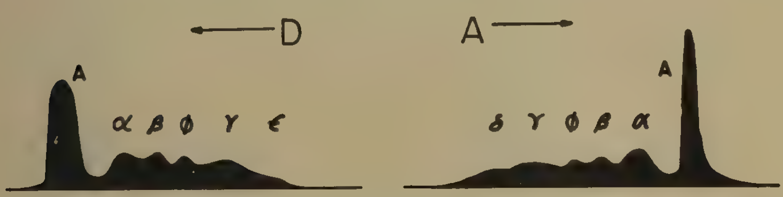

FIG. 1.—Normal electrophoretic pattern of adult female under the conditions used in this study.

All subsequent results are to be interpreted in view of the results of Table 2 as an indication of the reproducibility of the technique.

In Table 3 are summarized the results of sixteen analyses on eight individuals (Experiment 3). Variations are somewhat less than in Table 1, probably reflecting in part the improved technique. Nevertheless rather important individual variations exist. Thus the coefficient of variation for albumin (average of the two sides) is 7.0 as compared to only 1.2 for the replicate analyses on a single sample (Table 2). Fibrinogen would appear to be the next most variable component, alpha and beta globulin least variable.

Data on total protein (calculated from the dilution factor to give the final refractive increment of 0.0020) are also included in this table. This figure is subject to more experimental error than the electrophoretic analyses; thus in various experiments the coefficient of variation of replication of total protein appears to be in the range 3.3 to 8 per cent. It seems evident, nevertheless, that the total protein content is subject to much more dietary influence, at least over the range of diets studied here, than is the relative composition.

It should be pointed out that the results of the second bleedings in Experiment 3 are somewhat complicated by the fact that five of the gilts had been bred at the time of the second bleeding. It appears from these results that no appreciable change in composition has yet resulted (with the possible exception of gilt 8 which is anomalous). This point is covered in more detail in the following paper of this series.

In spite of the rather large degree of variability seen here the figures

for mean composition are in very good agreement with those obtained by Koenig and Hogness under similar conditions (3), namely albumin 38.1, α-globulin 18.8, β-globulin 13.4, fibrinogen 11.9, and γ-globulin 17.8 percent.

Finally it is of interest to consider the relative merits of using results from one or the other electrophoretic channel in work of this kind where reproducibility is of primary importance. It will be noted in Table 2 that in general using the average of the two sides yields greater precision than either side individually, though in some cases the advantage is not great. In Tables 1 and 3 it is seen that the average is frequently less precise than either the ascending or descending sides. In general the added work of tracing and planimetering both sides would hardly appear worthwhile. Also from the standpoint of reproducibility, it apparently matters little whether the ascending or descending pattern is used.

SUMMARY

Data are presented from thirty-eight electrophoretic runs on adult female swine plasma samples collected from twenty-six disease-free individuals on controlled nutritional planes. Precision of the electrophoretic technique is indicated by coefficients of variation ranging from only 1.3 per cent for albumin to 5.3 per cent for γ-globulin obtained from five replicate analyses on a single individual. Individual variations are relatively small but well outside the limits of experimental error. The average composition data are in good accord with those previously obtained on pooled samples. The relative merits of using ascending and descending patterns and averages of the two are discussed.

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NOTES ON SOME AMPHIBIANS AND REPTILES FROM DAVIS COUNTY, IOWA[1]

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Representatives of 25 species and subspecies of amphibians and reptiles were collected incidental to bob-white investigations in 1946, 1947, and 1948. The area under study is within sections 4, 5, 8, and 9 of Salt Creek Township, Davis County, Iowa. One-third of the 1,600-acre Eldon Research Area lies in the lowland bordering Salt Creek, a large tributary of the Des Moines River. This bottomland consists of small irregular tree-bordered fields and several marsh areas. The rough upland is cut by intermittent streams which are contaminated with drainage water from strip mines and hillside coal deposits. A large portion of the upland is covered with shrubs and third-growth timber, and the remainder is pastured, cultivated, or untilled because of erosion. Sixteen ponds of varying areas and depths occur in the upland.

The collection probably does not represent fully the herpetofauna of the area and does not include all the forms from Davis County in the Iowa State College collection.

Three species of turtles were identified in the field: snapping turtle, *Chelydra serpentina serpentina* (Linnaeus); painted turtle, *Chrysemys picta bellii* (Gray); and soft-shelled turtle, *Amyda spinifera spinifera* (LeSueur). The last named, common in Soap Creek, is a new record from Davis County.

The list of species follows. Specimens of all have been deposited in the Iowa State College collection. Forms marked with the asterisk are first accessions from Davis County.

Bufo terrestris americanus Holbrook. American Toad. Common.

Acris crepitans blanchardi Harper. Cricket Frog. Abundant.

* *Hyla crucifer crucifer* Wied. Spring Peeper. Single specimen, May 19, 1946.

Hyla versicolor versicolor LeConte. Common Tree Frog. Common.

Pseudacris nigrita triseriata Wied. Striped Swamp Frog. Most prevalent tree frog.

Rana catesbeiana Shaw. Bullfrog. Around permanent farm ponds only.

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[2] I am indebted to Dr. Reeve M. Bailey, Dr. Charles L. Walker, and Dr. Hobart M. Smith who examined specimens, and to the personnel of the Research Unit for suggestions concerning the manuscript.

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**Rana clamitans* Latreille. Green Frog. Single Specimen, July 11, 1948.

Rana pipiens Schreber. Leopard Frog. Abundant.

**Microhyla carolinensis carolinensis* Holbrook. Narrow-mouthed Toad. Single specimen, May 11, 1946. First record for Iowa [Klimstra (10)].

**Agkistrodon contortrix mokasen* Beauvois. Northern Copperhead. Single young specimen, along Soap Creek, August 26, 1948, fits Gloyd and Conant's (6) description of the southern subspecies in being pale with pinkish tinge, belly with a distinct series of ventrolateral blotches that alternate with the bases of the triangles formed by the hourglass-shaped cross-bands of the dorsal side. Also fits Bailey's (2) description of the northern form in number of scales, 2.5–3.5, in the cross-bands along the mid-dorsal line.

**Carphophis amoena vermis* Kennicott. Western Worm Snake. Two specimens, June 3 and 6, 1947, on plowed soil in cleared upland. One was typical *vermis*, but the other more nearly fitted the description of *amoena amoena* [Schmidt and Davis, (11)].

Coluber constrictor flaviventris Say. Blue Racer. Most abundant snake. The uterus of one specimen contained 19 eggs, June 3, 1946. Another specimen contained a 3– or 4–week old cottontail, *Sylvilagus floridanus mearnsii*, heavily fly-blown.

**Crotalus horridus horridus* Linnaeus. Timber Rattlesnake. Single specimen along an upland stream, August 30, 1948. Timber rattlesnakes were common in the rocky bluffs in Wapello County, less than five miles from the area.

**Diadophis punctatus arnyi* Kennicott. Western Ring-necked Snake. Single specimen, July 12, 1946, not typical *arnyi*. The neck ring was pale yellow and unbroken at the midline, and the belly was yellow, with a row of small imperfectly developed black spots down the center. This specimen is probably an aberrant form of *arnyi*, although it agrees somewhat with *edwardsi* as described by Schmidt and Davis (11).

**Elaphe obsoleta obsoleta* Say. Northern Pilot Blacksnake. Common, next to blue racer in abundance. Primarily in the upland along roads, cattle trails, dry stream beds, and around farm buidings. One, found in a hawthorn clump, *Crataegus* sp., measured 73 inches long. Eight others were between 60 and 66 inches long. Parents and young were observed in a partially filled road tile September 6, 1946, and August 29, 1947. The males were eight to ten inches shorter than the females. They strongly defended the site, while the females moved back and forth in front of the entrance to the nest.

Heterodon contortrix contortrix Linnaeus. Eastern Hog-nosed Snake. Third most abundant snake. Common in the upland, along roadways, dry stream beds, and field edges.

Lampropeltis calligaster Harlan. Prairie King Snake. Single specimen in bottomland near Soap Creek.

**Natrix grahamii* Baird and Girard. Graham's Water Snake. Single specimen in an upland road, May 31, 1946.

Natrix sipedon sipedon Linnaeus. Common Water Snake. Fourth in abundance.

**Opheodrys aestivus* Linnaeus. Keeled Green Snake. Single specimen collected from a coralberry bush, *Symphoricarpus orbiculatus,* in the upland—Klimstra, (8).

**Pituophis catenifer sayi* Schlegel. Prairie Bull Snake. Four individuals observed during 1946 and 1947, none in 1948.

**Storeria dekayi* Holbrook. De Kay's Snake. Single specimen, July 2, 1947, along an upland road.

**Storeria occipitomaculata* Storer. Red-bellied Snake. Single specimen, June 6, 1947, in a recently cleared upland area.

**Thamnophis radix* Baird and Girard. Plains Garter Snake. Single specimen, April 20, 1947, along a brushy intermittent creek in the bottomland.

Thamnophis sirtalis parietalis Say. Red-barred Garter Snake. Several individuals observed. One 13-inch specimen was noted attempting to swallow a common tree frog.

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THE POLAROGRAPHY OF VITAMIN B_{12}

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Of the various methods of determining the valence of the cobalt atom present in the molecule of vitamin B_{12}, polarography appears to be a quite direct approach and applicable to the small amounts of material available. That vitamin B_{12} is reduced at the dropping mercury cathode has already been reported briefly (1).

The coordination compounds of trivalent cobalt, as might be expected, show two polarographic waves (2,3) one in the region zero to —0.5 v. toward the saturated calomel electrode (s.c.e.) corresponding to the reduction of the trivalent cobalt to the bivalent stage, and a second wave, in the region —1.20 to —1.43 v. corresponding to the reduction of bivalent cobalt to the metal. The general ranges of the values for these two waves have been confirmed by unpublished work in this laboratory on the polarographic reduction of the trivalent cobalt derivatives of amino acids. Inasmuch as vitamin B_{12} contains some 14 nitrogen atoms and has an absorption spectrum having a general resemblance to that of cobaltic ammines, preliminary inspection of the problem indicates that B_{12} should be a chelate ring structure with at least a part of the involved coordination linkages being cobalt to nitrogen bonds and that it should show two reduction waves corresponding to those just mentioned.

EXPERIMENTAL WORK

MATERIALS

Three specimens of crystalline vitamin B_{12} were obtained from the E. R. Squibb and Sons Company of New Brunswick, N. J. Samples were weighed out directly and the weights corrected to a moisture-free basis, the moisture content of the samples being: A, 16.38 per cent; B, 15.03 per cent; C, 17.7 per cent. As a check in many cases aliquots of the solutions were accurately diluted and the transmittancy determined on the Beckman spectrophotometer at 361 mμ. The concentration of the original solution was then calculated using the value $E^{1\%}_{1cm} = 207$ reported by Brink *et al.* (4). Molar concentrations of the solutions were calculated both on the basis of the weight taken and on the spectrophotometric measurement, assuming the molecular weight of the B_{12} to be 1,500. The difference in the values for the concentration obtained

in these two ways is presumably a measure of the water or solvent of crystallization present in the B_{12}.

The tetramethylammonium bromide used was repeatedly recrystallized from water-alcohol mixtures. Tetramethylammonium hydroxide was prepared from the bromide by treatment with freshly precipitated and well washed hydrated silver oxide. Tetramethylammonium chloride was prepared from the hydroxide by treatment with hydrochloric acid prepared by passing hydrogen chloride into water. The water used was redistilled in tin equipment and stored in vycor flasks. The solutions of the tetramethylammonium compounds were stored in polyethylene bottles.

APPARATUS

The work was carried out with a Sargent Model XXI recording polarograph. Because of the great increase in drop rate at the highly negative potentials, a fine capillary and relatively low mercury head were used in the work with tetramethylammonium supporting electrolytes so that the drop time with no applied potential was quite great, about six seconds per drop. In the other measurements capillaries having drop times of three to five seconds were used. A simple cell of small volume was used. A commercial, saturated calomel reference electrode of small diameter, introduced into the solution through a hole in the stopper parallel to the capillary, was used as anode when potassium sulfate was used as the supporting electrolyte. There was sufficient leakage of potassium chloride through the asbestos fiber connection of this electrode so that the mercury anode was used when tetramethylammonium salts were employed as the supporting electrolyte; the potentials were then referred to the s.c.e. by a later measurement of the potential of the mercury anode toward the s.c.e.

The nitrogen used to sweep oxygen from the cell was freed of oxygen by passage through vanadous sulfate solutions (5) and then passed over ascarite to free it of any hydrogen sulfide occasionally formed in the vanadous sulfate-amalgamated zinc train.

Satisfactory polarograms of vitamin B_{12} were obtained in 0.1 M solutions of potassium sulfate at concentrations 10^{-5} to 10^{-3} M, for example, Figure 1. Other polarograms were made with 0.1 M tetramethylammonium hydroxide as the supporting electrolyte for the tetramethylammonium ion is reported (6) to be the most difficultly reducible of all ions. Polarograms were also made in solutions of tetramethylammonium hydroxide neutralized with hydrochloric acid to different values of pH as determined with a glass electrode apparatus. Still other polarograms were made in 0.1 M potassium nitrate, for the initial rise in current for this supporting electrolyte alone is about +0.4 v. in contrast to chloride and sulfate solutions in which the initial rise occurs about zero v. toward the s.c.e. The reduction of the nitrate ion at —1.5 v. makes this electrolyte unsuitable for studies in the far negative region but it

is apparently the best electrolyte for the region a few tenths volt positive to the s.c.e.

In general the polarograms were run at four sensitivity settings and parallel runs made on the supporting electrolyte alone.

The diffusion coefficient of vitamin B_{12} was measured by the method of Northrup and Anson (7,8) and also with the newer apparatus described by Stokes (9); the values found were 4.8×10^{-6} $cm^2 \cdot sec^{-1}$ (in water) by the former method and 4.44×10^{-6} (in water) and 4.46×10^{-6} (in 0.1 M potassium sulfate) by the latter.

RESULTS

No reduction wave was found for vitamin B_{12} in the region +0.3 to —1.0 v. toward the s.c.e. In all of the supporting electrolytes studied a reduction wave having a half-wave potential —1.12 v. toward the s.c.e. was found, Figure 1. Following this wave there was sometimes found

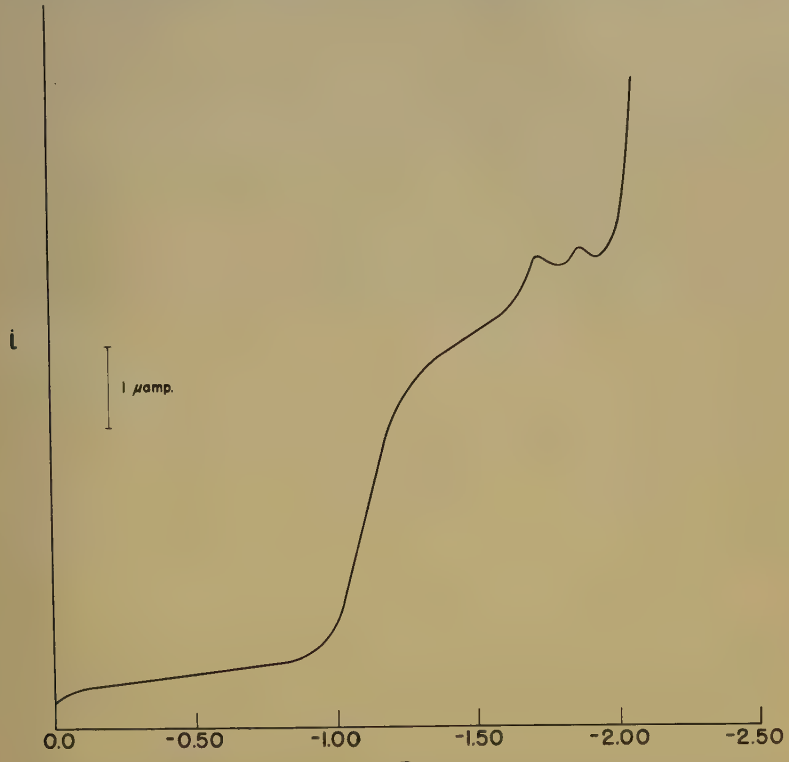

FIG. 1.—Polarogram of Vitamin B_{12}, specimen A, approximately 0.001 M in 0.100 M potassium sulfate, showing double maxima following first wave. Potential measured against saturated calomel electrode.

one or two maxima, the presence and size of which varied with the specimen of the vitamin examined and the concentration. At more negative potentials a large wave, many times the height of the first wave was found. Each of these characteristics of the polarogram of vitamin B_{12} will now be discussed in detail.

ABSENCE OF A WAVE IN THE REGION AROUND ZERO

Inasmuch as the cobaltic ammines show waves in the region zero to —0.5 v. particular attention was paid to detect any such wave of B_{12}. The polarogram of B_{12} in solutions containing potassium sulfate and tetramethylammonium chloride show no break but only a steadily rising residual current out to —1.0 v. They do have a slight rising portion at the start which suggests that a reduction wave might exist in the region just positive to the s.c.e. The polarograms of the two isomeric cobaltic glycine compounds are similar in showing this initial rise (although considerably larger) and an absence of any other reduction out to the region of the bivalent cobalt-cobalt reduction (half-wave potential —1.40 v. for the pink isomer and —1.51 v. for the purple isomer). In a 0.1 M potassium nitrate solution both of the cobaltic glycine compounds showed waves with half-wave potentials slightly negative to the s.c.e., —0.13 v. for the pink isomer and —0.28 v. for the purple isomer, the preceding residual current being well marked and lying in the region just positive to the s.c.e. The first wave in each case was just half the height of the later wave.

In 0.1 M potassium nitrate, however, B_{12} showed no such wave, the curves beginning at +0.5 v. superimposing exactly on those of the supporting electrolyte alone at the same sensitivity.

FIRST WAVE OF B_{12}

The half-wave potential of the first wave was essentially the same in each of the supporting electrolytes used: 0.1 M potassium sulfate, —1.12 v.; 0.1 M tetramethylammonium chloride, —1.11 v.; 0.1 M potassium nitrate, —1.15 v. toward the s.c.e. The half-wave potential of the first wave was also constant over the pH range studied (tetramethylammonium hydroxide-chloride mixtures): —1.19 v. toward the s.c.e. at pH 5.2, —1.11 v. at pH 6.9, —1.13 v. at pH 8.1, —1.10 v. at pH 9.4, and —1.10 v. at pH 11.7.

The reduction corresponding to this first wave is apparently irreversible. The slope of the wave in 0.1 M potassium sulfate supporting electrolyte was found to be 0.151 which would give an apparent value of about 0.39 for the number of electrons involved in the reduction. The waves obtained in tetramethylammonium hydroxide had a slope of 0.157 corresponding to a value of 0.375 for n. Such impossible values are usually interpreted as characteristic of an irreversible reaction (10).

More success was achieved in utilizing the Ilkovic equation for the

calculation of the number of electrons involved in the reaction. The results are summarized in Table 1. Even allowing for the uncertainty in the molecular weight there is little doubt that the reduction involves two electrons.

The variation of the diffusion current of the first wave is linear with concentration as determined spectrophotometrically at a wave length of 361 mμ. Below 5 x 10^{-5} M the wave merges with the residual current and cannot be measured satisfactorily; the upper limit was not determined, the greatest concentration studied being about 100 x 10^{-5}

TABLE 1

THE SLOPE AND NUMBER OF ELECTRONS INVOLVED IN THE FIRST REDUCTION WAVE OF VITAMIN B_{12}

Specimen	Supporting Electrolyte	n calc. from slope	Conc. Millimolar	Current amp.	n by Ilkovic Equation
Squibb C††	0.1 M K_2SO_4	0.393	8.33×10^{-1}†	3.21	1.56‡
Squibb C††	0.1 M K_2SO_4	0.391	8.33×10^{-1}†	3.51	1.71‡
Squibb B††	0.1 M K_2SO_4	0.375	9.68×10^{-1}†	3.87	1.63§
Squibb B	$Me_4NOH + Me_4NCl$**	0.375	8.70×10^{-1}*	3.48	2.12‖
Squibb B	0.1 M KNO_3	0.36	9.20×10^{-1}*	3.27	1.88‖

* Conc. determined by weight subtracting water reported present (15.03 per cent).
† Conc. determined spectrophotometrically.
‡ Capillary III; m = 1.71 mg. per sec., t = 5.84 sec. per drop.
§ Capillary IV; m = 1.69 mg. per sec., t = 4.77 sec. per drop.
‖ Capillary II; m = 1.074 mg. per sec., t = 6.04 sec. per drop.
** pH 11.7.
†† Crystallized from water by addition of acetone and vacuum dried over anhydrous magnesium perchlorate.

M. As an analytical method, the polarographic method is not as satisfactory as the spectrophotometric method; the slope of the curve beyond the wave is greater than the slope of the residual current so that there is always some uncertainty in the measurement of the diffusion current.

MAXIMA FOLLOWING THE FIRST WAVE

The two maxima seen in the polarogram of specimen Squibb A, Figure 1, were barely discernible in the polarogram of the second specimen, Squibb B. These double maxima (peaks at — 1.71 v. and — 1.86 v.) are the predominant feature of polarograms obtained in dilute solutions of the vitamin. At concentrations 5 x 10^{-5} M and lower the first wave can be barely distinguished from the general rise in current. The first

of these maxima has the same position as that in the polarogram reported by Fantes *et al.* (1). There appears to be no stoichiometric relation between the first, large wave and this maximum and it is assumed to be a catalytic or adsorption effect of some sort. The specimen designated Squibb C as well as Squibb C precipitated by acetone showed maxima at —1.83 v. and —1.99 v., both in potassium sulfate and in tetramethylammonium chloride. The —1.99 v. maximum was decreased by drying the material for ten days in a vacuum over anhydrous magnesium perchlorate. Neither the height nor the position of these maxima was changed by the addition of acetone to the solution. At lower concentrations of Squibb C these maxima were shifted to more positive positions and their relative sizes altered. At 10^{-5} M the first maximum lies about —1.7 v. and is large and the second has disappeared. This aspect of the polarogram of vitamin B_{12} probably cannot be explained until the structure of the molecule is known.

LARGE WAVE IN THE VERY NEGATIVE REGION

A second reduction wave of B_{12} if it existed would be obscured by the reduction of the potassium ion of the potassium sulfate used as the supporting electrolyte, the 45° tangent potential of 0.1 M potassium sulfate being around —1.9 v. (sensitivity 0.01 μ amp per mm.). The tetramethylammonium ion is reduced with much more difficulty, its tangent potential being reported (6) as —2.93 v. toward the s.c.e. We were unable to secure a tangent potential this negative for tetramethylammonium hydroxide, chloride or bromide, but inasmuch as the tangent potential changes with the current sensitivity this may have been due to our utilizing sensitivities higher than that used to obtain the value reported. At any rate by careful elimination of all sources of contamination by alkali metal ions we were able to secure a tangent potential for 0.1 M tetramethylammonium hydroxide-chloride mixture of —2.0 v., somewhat more negative than that found with potassium salts as the supporting electrolyte (sensitivity 0.03 μ amps per mm. of graph paper). Using such mixtures no second wave was found, the height of which was a simple multiple of the first. Rather, a huge increase in current was observed resembling the final current obtained in a polarogram when reduction of the supporting electrolyte begins. In this case, however, the tangent potential lay 0.2 v. more positive than that obtained with the supporting electrolyte alone. Parallel experiments in which the supporting electrolyte was made 0.001 M in potassium sulfate showed that the potassium wave was only 0.1 v. more positive than that for the supporting electrolyte so that the observed wave lies positive to that of potassium by 0.1 v. At pH 5.2 and 6.9 a definite diffusion wave was obtained following the rise in current so that the half-wave potential could be accurately determined, —1.79 v. and —1.80 v., respectively (measured at sensitivity 0.4 μ amp per mm.). This wave decreased in height with increasing pH and was detectable at pH 9.4 but did not appear at all at pH 11.7. At

the values of pH where the wave had a definite diffusion current, it was possible to adjust the sensitivity of the instrument so that both waves appeared on the same polarogram, see Figure 2. It is probable that this second large wave is the catalytic hydrogen wave reported by Heyrovsky and Babicka (11).

DISCUSSION

The absence of a reduction wave of B_{12} in the region +0.3 to —0.5 v., characteristic of the reduction of trivalent to bivalent cobalt in the cobaltic ammines, together with the appearance of a two-electron reduction at a half-wave potential of —1.12 v. in the general region of the bivalent cobalt to cobalt metal reduction makes it appear that the valence of cobalt in the B_{12} molecule is two. However, there are several facts which indicate that the matter may not be quite so simple.

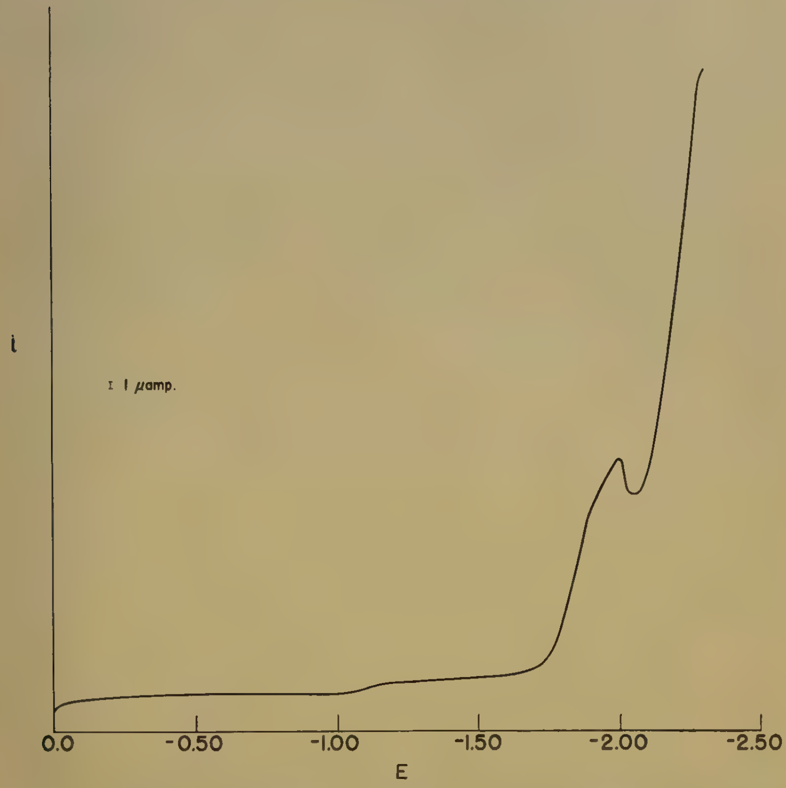

FIG. 2.—Vitamin B_{12}, specimen B, about 6.5×10^{-4} M, in 0.100 M tetramethylammonium hydroxide-tetramethylammonium chloride, pH 5.20, sensitivity greatly reduced to show relative heights of the two waves.

The hydrogenation of B_{12} leads to a brown product which on exposure to air is converted to a material, designated B_{12a}, closely resembling B_{12} (12); this reoxidation undoubtedly involves cobalt and it is apparent therefore that the reduction cannot involve the formation of metallic cobalt for reoxidation would then be impossible. Vitamin B_{12} was found (13) to be diamagnetic which can only be the case if the cobalt is trivalent or bivalent and coupled with molecular oxygen. The trivalent to bivalent cobalt wave being absent, it becomes pertinent to look into the possibility of B_{12} being an oxygenated bivalent cobalt compound.

A few compounds have the property of absorbing and releasing oxygen reversibly: bi-(disalicylalethylenediimine)-μ-aquodicobalt (14), a solution containing cobaltous chloride, ammonium chloride and ammonia (15), and the cobaltous derivatives of certain amino acids, notably histidine (16). In these materials the cobalt is bivalent and the oxygenated material diamagnetic. The combination of cobalt and oxygen is $2\,Co:O_2$ and the oxygen presumably links two cobalt atoms together in the form of a peroxo group Co–O–O–Co. The reduction of oxygen combined in this manner would resemble the reduction of hydrogen peroxide.

$$H_2O_2 + 2\,H^+ + 2\,e^- = 2\,H_2O$$
$$RCo{-}O{-}O{-}CoR + 4\,H^+ + 4\,e^- = 2\,RCo + 2\,H_2O$$

The reduction wave of hydrogen peroxide is drawn out but has a half-wave potential of —0.9 to —1.0 independent of pH but varying somewhat with the buffer used (17). The reduction observed for B_{12} resembles the reduction of hydrogen peroxide in having a half-wave potential in about the same region, in involving two electrons (per cobalt), in being irreversible, and in being independent of pH. The oxygen-carrying cobaltous compounds just mentioned give up their oxygen, at least in part, to an oxygen-free atmosphere. However, prolonged bubbling of oxygen-free nitrogen through a solution of B_{12} either at room temperature or at 50° did not alter the polarogram. It was observed that the oxygen is removed more slowly from a solution of B_{12} than from the supporting electrolyte alone.

The alternative to an oxygenated bivalent form for the cobalt of vitamin B_{12} is trivalent cobalt with the —1.12 v. wave representing the reduction of the trivalent state to the univalent state. The only univalent cobalt compound known appears to be that formed in the reduction of the pentacyano compounds of tri- and bivalent cobalt as reported by Hume and Kolthoff (18). Univalent cyanides of nickel and palladium, $K_2Ni(CN)_3$, $K_2Pd(CN)_3$, and even a zero valent nickel cyanide, $K_4Ni(CN)_4$, are known (19). The existence of such compounds probably depends on a number of factors of which an important one must be the strength of the metal-cyanide bond. That cobalt is firmly attached to the organic portion of the molecule of B_{12} is evident from the chemical behavior of the vitamin. On this score then the chelate-ring

structure about the cobalt must be sufficiently strong to make possible a stable univalent cobalt compound.

SUMMARY

Vitamin B_{12} has been studied with the polarograph. It displays a well defined reduction wave having a half-wave potential of —1.12 v. toward the saturated calomel electrode.

By means of the Ilkovic equation and a measured value of the diffusion constant of vitamin B_{12}, the wave has been shown to be due to a two-electron reduction; however, the slope of the wave indicates that the reduction is irreversible. The value of the half-wave potential is independent of pH over the range 5.2 to 11.7 and has essentially the same value in the supporting electrolytes potassium sulfate, tetramethylammonium hydroxide, tetramethylammonium chloride and potassium nitrate. The diffusion current of the wave is linear with the concentration of B_{12} as determined spectrophotometrically.

The wave is followed by one or two maxima which vary in position and height with the source of the material and with concentration and which are not stoichiometrically related to the concentration.

The observed wave is undoubtedly associated with the cobalt in the molecule. Cobaltic ammines in general show two reduction waves. The first, in the region zero to —0.5 v., corresponds to the reduction of trivalent cobalt to bivalent cobalt; a wave in this region, however, is missing with B_{12} and moreover it does not appear in the region zero to +0.4 v. as shown by polarograms in a solution of potassium nitrate. The second wave of the cobaltic ammines, a two-electron reaction corresponding to the reduction of bivalent cobalt to the metal, falls in the region —1.2 to —1.5 v. The reduction wave of B_{12} falls somewhat positive to this but would indicate that the cobalt in B_{12} is bivalent. Such a valence state is difficult to reconcile with certain other chemical and physical properties of vitamin B_{12} and two alternatives are suggested.

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